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VOLUME 4 NUMBER 3

PLANT PHYSIOLOGY

JULY, 1929

CONTENTS

On Some Limiting Factors in the Use of Saturated Petroleum Oils as Insecticides. HUGH KNIGHT, JOSEPH C. CHAMBERLIN, and CHARLES D. SAMUELS 299

Opening of Stomata in Different Ranges of Wave Lengths of Light. J. D. SAYRE. Ohio Agricultural Experiment Station 323

Mechanism of Cell Wall Formation. O. L. SPONSLER. University of California 329

The Pure Pigments, Carotin and Xanthophyll, and the Tswett Adsorption Method. F. M. SCHERTZ. United States Department of Agriculture 337

Growth Studies on Fruits. Respiration of Tomato Fruits. FELIX G. GUSTAFSON. University of Michigan 349

Some Observations on the Microchemical Demonstration of Phloridzin. E. M. HARVEY. Oregon Agricultural Experiment Station 357

The Effect of pH Value and Hydrogen Peroxide Concentration on Fruit Oxidase Activity. W. V. CRUESS and W. Y. FONG. University of California 363

Testing the Sugar Content of Beets for Genetical Purposes. ERNEST REED. Syracuse University 367

Effect of Acid and Alkaline Hydrolysis on the Estimation of Hemicelluloses and Associated Groups in Young Apple Wood. FISK GERHARDT. Iowa Agricultural Experiment Station 373

Influence of Shape on the Chemical Composition of Potato Tubers. J. J. WILLAMAN and ALICE M. CHILD. Minnesota Agricultural Experiment Station 385

Brief Papers. The Water Content of Wheat at Flowering Time. ANNIE M. HURD-KARRER and J. W. TAYLOR. United States Department of Agriculture 393

Notes 399

PUBLISHED QUARTERLY
BY
THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS
AT LIME AND GREEN STREETS, LANCASTER, PA.
THE SCIENCE PRESS PRINTING CO.

Entered as second-class matter March 25, 1926, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

PLANT PHYSIOLOGY

A QUARTERLY JOURNAL
DEVOTED TO ALL PHASES OF PLANT PHYSIOLOGY

Established 1926

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Subscription price, $8.00 a year. Single copies, $2.50 post free. Members of the American Society of Plant Physiologists receive the journal in connection with dues paid to the Society.

Missing numbers will be supplied if lost in the mail, provided that written notice is received within one month of date of issue. Otherwise they will be furnished at the single copy rate, as long as they are available.

Remittance should be made payable to PLANT PHYSIOLOGY.

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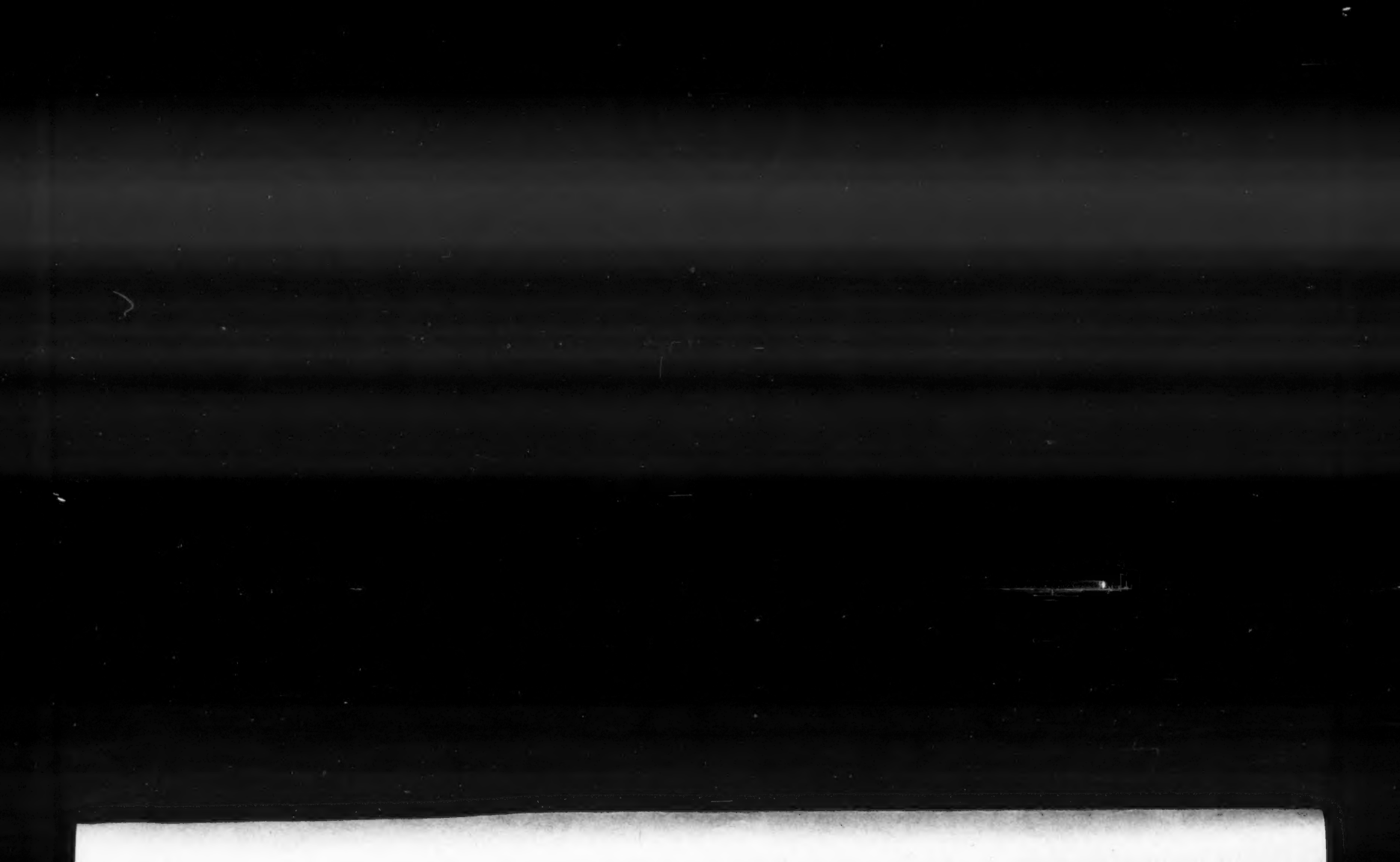

Vol. 4 No. 3

PLANT PHYSIOLOGY

JULY, 1929

ON SOME LIMITING FACTORS IN THE USE OF SATURATED PETROLEUM OILS AS INSECTICIDES[1]

HUGH KNIGHT,[2] JOSEPH C. CHAMBERLIN,[2]
AND CHAS. D. SAMUELS

(WITH TWO FIGURES)

Introduction

Owing to the increased use of saturated petroleum oils (*i.e.*, petroleum oils from which all, or nearly all *unsaturated hydrocarbons* have been removed, usually 98 per cent. or more) as insecticides when applied to foliage-bearing fruit-trees, it has become important to study the effects thus produced on the host plant.

The insecticidal efficacy has already been established by DE ONG and the two senior authors of this paper (**2**). It was noted that these oils apparently produce some adverse physiological effects on citrus trees, but this phase of the problem was not especially investigated.

The general nature of the deleterious effects accompanying the use of *heavy*, white-oil sprays has recently been described by WOGLUM, LA FOLLETTE and LANDON (**6**) as follows:

"The bad effects which have been noted on oranges in some degree since highly refined heavy oil sprays have been used are numerous. They include retarding of blossoming; reduction of blossoming; reduction of crop; retarding fruit coloration; interfering with normal sweat room coloring; drop of immature fruit; drop of mature fruit; roughened texture; mummifying of fruit; increasing crystallization; producing insipid flavor;

[1] The investigation reported as Part I of this paper was conducted by KNIGHT and SAMUELS, Part II by KNIGHT, and Part III by CHAMBERLIN. The work was done while the authors were in the employ of the California Spray-Chemical Company, of Watsonville, California. The studies reported on in Part III were made independently.

[2] Formerly Assistants in Entomology, Citrus Experiment Station, University of California, Riverside, California.

decrease in acidity, soluble solids and sugars; increase of dead wood; occurrence of fruit burn and leaf drop; difficulty in cleaning fruit, and "gumming-up" packing house conveyors. . . . Some of these troubles were costly to the grower. For instance extreme application of the heaviest oil sprays on Valencias during October and November, in some cases reduced the succeeding crop as much as 50 per cent., and affected the quality of the fruit then on the trees."

Not all of these effects have been fully verified. Some of them are established in the discussion which follows. It should be emphasized that these effects result primarily from the application of *heavy oils* (*i.e.*, saturated lubricating oils of high viscosity).

The quick-breaking white oil sprays were developed particularly with reference to California citricultural conditions and it is there that they have been most extensively used. As insecticides they are markedly successful and because of the decreasing efficacy of HCN fumigation in California due to the development over wide and increasing areas of HCN resistant scale (**5**), heavy oil sprays have rapidly come into use. The practical need of a substitute for present methods of HCN fumigation is not likely to lessen, but the results herein presented indicate that this substitute should be something other than a *heavy* petroleum oil spray. Our data emphasize the extreme importance of a knowledge of the metabolic and pathological results following the use of oil sprays.

Part I

Some notes on the physiological effects of saturated, white petroleum oils on Citrus

In order to ascertain something of the metabolic changes induced in the plant by the application of petroleum oil as insecticides, the following experiments were made. The problem was attacked from three angles, namely transpiration, photosynthesis and respiration. The plants used were "seed-bed stock" of sour orange (*Citrus aurantium* Linn.).

Transpiration.—The plants for the transpiration tests were taken from two nurseries, one in Riverside, California, and one in Glendora, California, and were about 25 centimeters in height. Preliminary tests showed that the source of the plants is important. Those from the Riverside nursery taken in August had an average weekly transpiration rate of 40 grams of water per square decimeter of leaf surface, while plants, apparently just as thrifty, taken from Glendora, where cultural conditions were not the same, gave a weekly average of 21 grams per same unit area. Plants from the Riverside nursery taken in February showed a transpiration rate of 12.5 gm. per week. This indicates that the transpiration rate varies greatly with differences of environment—cultural, climatic and seasonal.

Two sets of experiments were run, the first between August and October, 1926; the second between February and May, 1927. The data are presented in tables I and II. The experimental conditions, aside from season, were identical in the two cases. The plants were grown in sealed jars containing HOAGLAND'S nutrient solution. The weekly transpiration losses were replaced with distilled water. The leaf surface of each plant was measured and the loss of solution determined by daily weighings. The average transpiration loss for the two weeks prior to the application of the oil was taken as a norm for purposes of comparison. All results are expressed in two forms: first, in grams of water transpired per week per square decimeter of leaf surface; and secondly, as percentage of the "normal" rate as determined prior to treatment. Individual variations between plant and plant are considerable and differences of less than fifteen to twenty per cent. can not be considered significant. The data presented in any one vertical column are comparable, but those in different vertical columns are not, owing primarily to fluctuations in humidity.

Both humidity and temperature records were kept for the first test (table I). They are not included for the reason that no significant correlation could be found. Within the normal range of variation temperature alone is not significant, for, if the ratio of relative humidity to temperature remains constant, there is no significant change in transpiration. When the relative humidity drops, however, there is an immediate increase in transpiration. But since the change in transpiration caused by weather conditions was constant for all lots included in the tests, it may be disregarded in the present connection.

The application of oil causes a sharp and abnormal drop in the transpiration rate, the extent of the disturbance varying directly with the viscosity of the oil. In the case of kerosenes and light lubricating oils, recovery is effected in from one to three weeks. With saturated oils of high viscosity (100–110 seconds SAYBOLDT) recovery does not begin under six or seven weeks and may be delayed much longer than that. The length of time required for recovery is also correlated with the amount of oil applied. It is more rapid where the oil has been applied in small amounts (*i.e.* when applied as an emulsion), than where it is applied in large amounts (*i.e.* painted on in pure form). As indicated by experiment no. 12, table I, the presence of even a small percentage of unsaturates in an oil is sufficient to cause a sharp drop in the transpiration rate and to postpone recovery materially.

PHOTOSYNTHESIS AND RESPIRATION.—To determine the extent to which photosynthesis was affected by an application of oil, a thrifty potted sour orange plant somewhat more than 60 centimeters in height was kept in a dark chamber for fifteen days, when the leaves gave no further starch

TABLE I

THE EFFECTS OF PETROLEUM OIL ON THE TRANSPIRATION OF SOUR ORANGE NURSERY STOCK (AUGUST TO OCTOBER, 1926)

No. of experiment	General specifications of oil[1]	Mode of application	Transpiration rate at start of test[2]	Transpiration rate per week after application of oil. Weeks numbered 1–8[3]							
				1	2	3	4	5	6	7	8
1	Saturated; white petroleum; viscosity 106	Both sides of leaf painted with pure oil	38.9 gm. per week	15.3 gm.	15.7 gm.	18.4 gm.	14.3 gm.	12.3 gm.	12.6 gm.	18.1 gm.	22.3 gm.
			100 per cent.	39 per cent.	40 per cent.	47 per cent.	37 per cent.	32 per cent.	32 per cent.	47 per cent.	57 per cent.
13	Same as above	Same as above	46.7 gm. per week	12.2 gm.	28.0 gm.	25.7 gm.	30.8 gm.	31.3 gm.	38.0 gm.	45.3 gm.	.....
			100 per cent.	26 per cent.	60 per cent.	55 per cent.	66 per cent.	69 per cent.	81 per cent.	97 per cent.	.....
11	Saturated; white petroleum; viscosity 50	Same as above	34.4 gm. per week	27.7 gm.	27.2 gm.	31.2 gm.	27.7 gm.	.....	.....	.....	.....
			100 per cent.	81 per cent.	79 per cent.	91 per cent.	81 per cent.	.....	.....	.....	.....
6	Same as above	2 per cent. quick-breaking emulsion	30.1 gm. per week	16.1 gm.	35.7 gm.	.....	.....	.....	.....	.....	.....
			100 per cent.	54 per cent.	119 per cent.	.....	.....	.....	.....	.....	.....
9	Highly refined nearly saturated kerosene	Both sides of leaf painted with pure oil	43.5 gm. per week	29.8 gm.	51.3 gm.	44.0 gm.	.....	.....	.....	.....	.....
			100 per cent.	68 per cent.	118 per cent.	101 per cent.	.....	.....	.....	.....	.....
12	80 per cent. saturated; lubricating petroleum; viscosity 45	Same as above	31.5 gm. per week	12.3 gm.	18.1 gm.	18.8 gm.	11.0 gm.	15.6 gm.	11.4 gm.	9.8 gm.	8.1 gm.
			100 per cent.	39 per cent.	57 per cent.	60 per cent.	35 per cent.	50 per cent.	36 per cent.	31 per cent.	25 per cent.
10[4]	None	Check; no treatment	34.2 gm. per week	.....	.....	39.2 gm.	49.0 gm.	40.2 gm.	29.4 gm.	37.6 gm.	21.7 gm.
			100 per cent.	.....	.....	114 per cent.	143 per cent.	117 per cent.	86 per cent.	109 per cent.	63 per cent.

[1] Degrees of saturation and viscosities are fairly close approximations. Viscosities are given in seconds SAYBOLT at 100° F.
[2] Average rate as determined by two weeks' observation, in grams of water per square decimeter of leaf surface per week.
[3] Percentages to nearest whole per cent. only.
[4] Owing to an accident in setting up, there are no readings for the check for the first two weeks following the oil treatments.

[1] Degrees of saturation and viscosities are fairly close approximations. Viscosities are given in seconds SAYBOLT at 100° F.
[2] Average rate as determined by two weeks' observation, in grams of water per square decimeter of leaf surface per week.
[3] Percentages to nearest whole per cent. only.
[4] Owing to an accident in setting up, there are no readings for the check for the first two weeks following the oil treatments.

TABLE II

THE EFFECTS OF PETROLEUM OIL ON THE TRANSPORTATION RATE OF CITRUS (SOUR ORANGE) NURSERY STOCK (FEBRUARY TO MAY, 1927)[1]

NO. OF EXPERIMENT	GENERAL SPECIFICATIONS OF OIL	MODE OF APPLICATION	TRANSPIRATION RATE AT START OF TEST	TRANSPIRATION RATE PER WEEK AFTER APPLICATION OF OIL. WEEKS NUMBERED 1–8							
				1[2]	2	3	4	5	6	7	8
21	Saturated; white petroleum; viscosity 106	2 per cent. quick-breaking emulsion	10.4 gm.	2.0 gm.	2.4 gm.	4.5 gm.	9.1 gm.	6.1 gm.	7.0 gm.	15.8 gm.	.....
			100 per cent.	19 per cent.	23 per cent.	42 per cent.	87 per cent.	59 per cent.	62 per cent.	152 per cent.	.....
26	Same as above	Same as above	16.7 gm.	5.9 gm.	4.2 gm.	5.6 gm.	9.8 gm.	7.0 gm.	8.4 gm.	21.0 gm.	.....
			100 per cent.	35 per cent.	25 per cent.	33 per cent.	59 per cent.	42 per cent.	50 per cent.	125 per cent.	.....
24	None	Check; no treatment	11.8 gm.	7.3 gm.	14.0 gm.	13.3 gm.	11.7 gm.	10.5 gm.	12.6 gm.	9.8 gm.	13.6 gm.
			100 per cent.	62 per cent.	118 per cent.	112 per cent.	99 per cent.	89 per cent.	107 per cent.	83 per cent.	115 per cent.
37	None	Check; no treatment	15.7 gm.	8.1 gm.	16.3 gm.	16.6 gm.	14.6 gm.	11.7 gm.	15.9 gm.	11.1 gm.	15.3 gm.
			100 per cent.	52 per cent.	104 per cent.	106 per cent.	93 per cent.	74 per cent.	101 per cent.	71 per cent.	97 per cent.

[1] See footnotes 1, 2, 3, table I, which likewise apply to the data in this table.
[2] Initial low transpiration during the first week following treatment due to humid weather. Note figures for checks.

reaction to the iodine test. In the evening of the fifteenth day the plant was placed in very subdued light and was treated as follows:

Several leaves were painted on both surfaces with (1), a saturated white oil of 106 seconds viscosity; (2), with a saturated white oil of 50 seconds viscosity; and, (3) with a nearly saturated, highly refined kerosene oil. The treated leaves were then tagged and the plant returned to the dark chamber till the following evening when it was put outside, hence 36 hours lapsed before exposure to sunlight.

Sections were cut from the treated leaves daily and tested for starch. It was not until the fourteenth day that those of lot 1 gave a faint reaction to starch, and they *had not returned to normal on the fortieth day when the test was terminated.* Lot 2 showed a faint starch reaction on the first day and had returned to normal by the fourth. Lot 3 gave starch reaction the first day. Checks gave a normal starch reaction the first day.

In order to determine the extent to which photosynthesis was interfered with under orchard conditions, analyses of the starch content of leaves taken from a lemon grove which had been sprayed with a white, saturated petroleum oil emulsion (viscosity 106 seconds SAYBOLDT) at 2 per cent. concentration, were made at approximately regular intervals. The results are set forth in table III. These lemon trees were sprayed on December 15, 1926. Surrounding lemon groves were fumigated. Leaf samples were taken at random from both the sprayed and fumigated trees, those from the fumigated trees being taken at the beginning and at the end of the experimental period. These latter may be considered as a check. The

TABLE III

STARCH ACCUMULATION IN LEMON LEAVES SPRAYED WITH A SATURATED WHITE PETROLEUM OIL SPRAY
(VISCOSITY 106)
(FIELD CONDITIONS)

NATURE AND DATE OF TREATMENT	GRAMS OF STARCH IN 5 GRAMS OF LEAVES	PER CENT. OF STARCH TO DRY WEIGHT OF TREATED LEAVES	DATE SAMPLED
	gm.	*per cent.*	
Fumigated with hydrocyanic acid gas, December 15, 1926	0.0986	1.97	Dec. 29, 1926
	0.0960	1.92	Feb. 25, 1927
Sprayed with white oil emulsion at 2 per cent. concentration, December 15, 1926	0.0910	1.92	Dec. 29, 1926
	0.0744	1.49	Jan. 12, 1927
	0.0566	1.13	Jan. 21, 1927
	0.0604	1.21	Feb. 8, 1927
	0.1160	2.32	Feb. 25, 1927

results show that the starch content of the unsprayed trees remained constant within the limits of normal variability and experimental error. In the sprayed block the starch content dropped off markedly the first five weeks, and then rose rapidly until at the termination of the test it was far in excess of that of the check trees. Results similar to the above, showing increased starch content in the leaves two or more months after spraying have been noted in several other groves.

An attempt was also made to determine the effect on respiration, by the application of a heavy white oil as follows:

The above-ground portion of a potted Citrus plant was sealed under a large bell-jar. A small opening was provided at the bottom for the inflow of air, and a second small opening at the top to permit samples to be drawn off. The air taken out at the top was run through a gas train which removed the moisture and then the carbon dioxide was trapped in soda-lime and weighed. The amount of air passing through the system was controlled by means of an aspirator connected to the train. In this way the volume of water flowing from the aspirator was equal to the volume of air entering the system. Thirty-six liters of air were drawn through the apparatus for each determination. Several determinations were made with the empty apparatus to determine the amount of CO_2 normally present in the air. This was found to be 0.0166 grams per 36 liters. It is evident that when the same amount of air is drawn through the apparatus with the plant enclosed and more than this amount of CO_2 is found, that the plant is giving off CO_2, or, in other words is respiring in excess of photosynthesis. The point at which these two quantities are equivalent is indicated by line "A" in fig. 1, where the results of this test are shown in graphic form. The distance of the plotted curve above or below this line therefore shows the extent to which the one or the other of these processes predominates.

Two thrifty untreated Citrus trees about six decimeters in height were placed under bell jars as before indicated and the CO_2 value was determined for each on four consecutive days. The curve (fig. 1) is well below line "A" and shows that photosynthesis was therefore in excess of respiration, the plants fixing an average of ten milligrams of CO_2 each day during the period covered by the test. Each run lasted approximately five and one-half hours. The four-day average is indicated in fig. 1 by the broken line designated "Norm." This is to be considered an average of photosynthetic activity for the plants in question, only for this season of the year; it would probably be greatly exceeded during the growing season.

The plants were then treated as follows: The entire leaf surface of one was painted with saturated oil of 106 seconds viscosity. The other was sprayed with a 2 per cent. emulsion of the same oil.

The graph, fig. 1, is self-explanatory. Respiration was enormously increased, the plants evidently oxidizing great quantities of reserve food.

Neither plant had returned to normal at the expiration of a month after the application of the oil. The reaction of the two plants differed only in degree, as was to be expected.

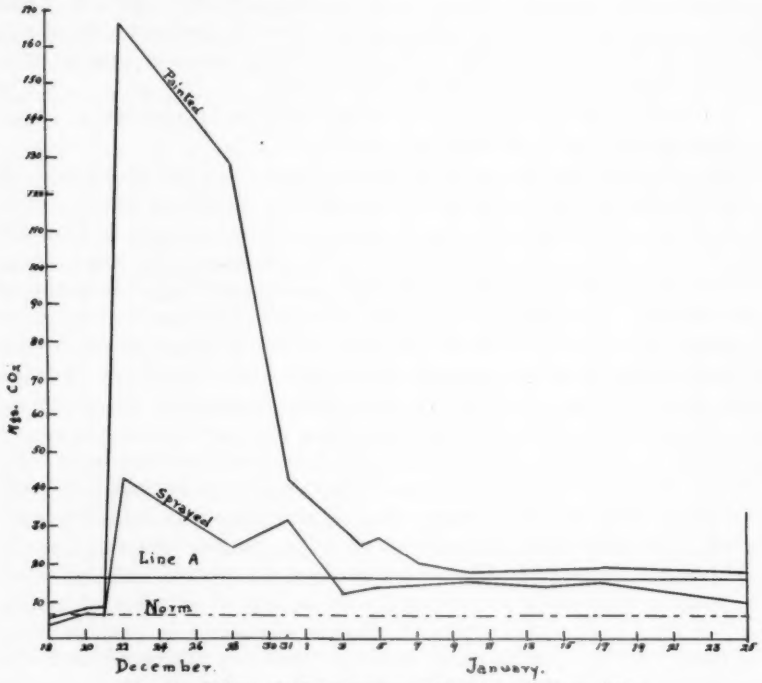

FIG. 1. Effect of oil sprays on respiration of Citrus trees.

It is recognized that the data presented are not sufficient to furnish a basis from which to draw definite conclusions. They indicate, however, that the application of a viscous, saturated petroleum oil induces profound metabolic changes in Citrus trees which persist for an extraordinary length of time.

It seems desirable to emphasize the fact that mere lack of "corrosive" qualities or the possession of "neutrality" or "chemical inertness" on the part of an oil does not imply that it is without deleterious effects when applied to living plants.

Part II

THE TRANSLOCATION OF PETROLEUM OIL IN THE LIVING PLANT (CITRUS)

Part I of this paper presented in a general way some of the physiological effects produced on the metabolism of *Citrus* by an application of a highly

refined, saturated petroleum oil, and indicated that the inhibitory effects are of surprisingly long duration.

Field observations also indicate that the heavier oils are absorbed by the leaves and remain therein for a very long period of time. The experiments already presented show that the functional activity in the leaf is partially resumed within about sixty days. In order to ascertain, if possible, how the oil was finally disposed of by the plant a histological study of oil penetration into the leaf was undertaken. To accomplish this successfully it was necessary to devise a special technique for staining the oil within the leaf tissue. It was recognized at the outset that none of the solvents in general use could be employed, as they are all oil solvents; and the problem was to fix or clear the leaf without dissolving the oil. Consequently the use of alcohol, xylol, or any of the essential oils as well as canada balsam was precluded, as was also the paraffin method of preparing sections.

Two kinds of preparations were desirable, (1) a flat gross preparation in which the distribution of oil in the leaf could be observed over a relatively large area, and (2), cross-sections in which the individual cells could be observed in their relation to the absorbed oil thus securing some insight into the penetration of oil into the cells themselves.

For the gross preparations it was necessary to "clear" the leaf, that is, to dissolve the chlorophyll and render the leaf transparent without dissolving or disturbing the oil; and for cross-sections, to fix the tissues for staining and sectioning also without disturbing the oil.

A short note in "Stain Technology" (**4**) gave a suggestion which materially helped to solve both phases of the problem. This note called attention to the use of pyridine in aqueous solution as a carrier for a fat stain. Pyridine is soluble in water as well as oil. Both Sudan III and IV, and Oil Soluble Red O are soluble in pyridine. When an oil-treated leaf is immersed in a saturated solution of Oil Soluble Red O dissolved in an aqueous pyridine solution, the stain, having a greater affinity for the oil than for the aqueous pyridine solution, is taken up by the oil. The solution of pyridine can then be removed by washing. In addition it was found that an aqueous solution of pyridine is an excellent solvent for chlorophyll, thus permitting "the killing of two birds with one stone" for it thereby became possible to clear the leaf and stain it at the same time without disturbing the oil.

The technique developed is substantially as follows:

For flat gross preparations.—Immerse in sixty per cent. aqueous solution of pyridine. Heat over water-bath. When discolored pour off and refill with fresh solution. Repeat (usually twice is sufficient) till solution remains clear and specimen becomes transparent.

Immerse for twenty-four hours in saturated solution of Oil Red O dissolved in 70 per cent. aqueous solution of pyridine.

Differentiate in 50 per cent. pyridine.

Wash in running water.

Pass through (1) glycerine-water (equal parts), followed by (2) pure glycerine.

Clear in carbol-glycerine (1 part carbolic acid to 2 parts glycerine). Heat gently and watch carefully under dissecting microscope till clear. Specimen should be turned under-side up, when oil droplets can be seen distinctly.

Pass through glycerine again and mount in glycerine-jelly.

Allow to harden and seal with clear "Duco."

For cross-sections.—Fix in chrome-acetic acid for 48 hours.

Wash in running water.

Immerse in 5 per cent. formalin for 30 minutes.

Wash again in running water.

Immerse in 50 per cent. pyridine (aqueous solution) for 10 minutes.

Stain for twenty-four hours in saturated solution of Oil Red O dissolved in 70 per cent. aqueous solution of pyridine.

Differentiate in 50 per cent. aqueous solution of pyridine until color ceases to stream (watch carefully).

Wash in running water.

Section. See below.

Pass through (1) glycerine-water, followed by (2) glycerine.

Mount in glycerine-jelly and seal with clear "Duco."

Inasmuch as imbedding in paraffin is precluded, cross-sections were made by means of pith or cork. The freezing method could no doubt be used, but the writer has not had the opportunity of trying it. The operations of staining, washing, clearing and so on, are carried on with the aid of watch-glasses or small shell vials. If watch-glasses are used for staining they should be placed in closed petri dishes to prevent excessive evaporation. Watch-glasses are preferable for use with cross-sections. Staining may be done either before or after sectioning. If done before, shell vials will be found preferable.

For purposes of comparison the following oils were used:

(1) Heavy, saturated, white petroleum oil of 100–110 seconds viscosity SAYBOLDT at 100° F.

(2) A medium lubricating oil consisting of an equal mixture of 1 and 3. Saturation about 97 per cent.; viscosity about 67.

(3) Light, lubricating petroleum oil of about 50 seconds viscosity and 96 per cent. saturation.

(4) Light, lubricating oil of 44 seconds viscosity and 67 per cent. saturation; an unsaturated oil probably blended with a distillate.

It is regrettable that accurate data regarding other physical constants of these different oils are not available, such as vapor pressure at ordinary temperature, acidity, rate of oxidation, etc. Until such information is available much of the value of this type of study is lost.

Experimental data

Four potted orange plants were selected, of uniform size (about 6 decimeters in height) and having one or two lateral branches. Several of the leaves, from the tip to center of one limb, were carefully treated with oil applied by means of a small artist's brush, the oil being applied to both surfaces of the blade as far back as the petiole. The limb was tagged at the base of the lowest treated leaf. After treatment the plants were set away in the lath house.

Oil number 1 was taken as an index for the initial penetration tests, samples being cut from the leaves at intervals of 1, 2, 4, 8 and 24 hours. Owing to the great amount of labor involved, it was not possible to take samples from all four treated plants for this initial test. This is unimportant, the only possible difference being one of rapidity of penetration, the lighter oils penetrating more rapidly. Oil number 3, for example, disappeared from the surface in approximately three days, while oil number 1 remained a week or more. (See also the data on penetration in part III of this paper.)

At the expiration of the first twenty-four hours and at certain intervals afterwards, samples were taken from all four plants simultaneously and were treated together, each sample being cut to a distinctive pattern to facilitate its recognition.

After the first 24 hours, samples were taken every three days until it became evident that the withdrawal or disappearance of oil from the tissue of the leaf, even in the case of the lighter oils, was a very lengthy process. The time between samplings was then lengthened to one week.

The disappearance of oil, either from the surface or from within the leaf, was not caused by evaporation, as is generally supposed, except perhaps in a very minor degree. As a matter of fact, the evaporation of oils of high boiling points and low vapor pressures (lubricating oils) is probably a negligible factor at ordinary atmospheric temperatures, especially when enclosed within the intercellular spaces of the leaf tissue. Only those oils with boiling points within or near atmospheric temperature ranges evaporate quickly, *i.e.*, benzine, gasoline, kerosene, etc.

Each set of samples was prepared and mounted both in flat gross mounts and cross-sections, carefully studied under the microscope and compared with preceding lots.

Manner of penetration

Samples taken at the expiration of one hour after treatment showed that the cutinized outer integument covering the epidermal cells absorbs oil rapidly. On the upper surface of the leaf the oil accumulates in the depressions between the cells as shown in fig. 2 A. On the lower epidermis it pours through the stomata and runs along the cell walls, frequently coalescing into droplets between the outer walls of the guard cells (fig. 2 B, C). Such droplets disengage themselves and pass into the intercellular spaces of the spongy chlorenchyma. When drops are not formed at this point the oil may run along the outer surface of the epidermal cell wall and gradually spread out between the cells of the spongy chlorenchyma.

At the end of the second hour this process shows further developments. A considerable amount of oil now shows along the outer surface of the

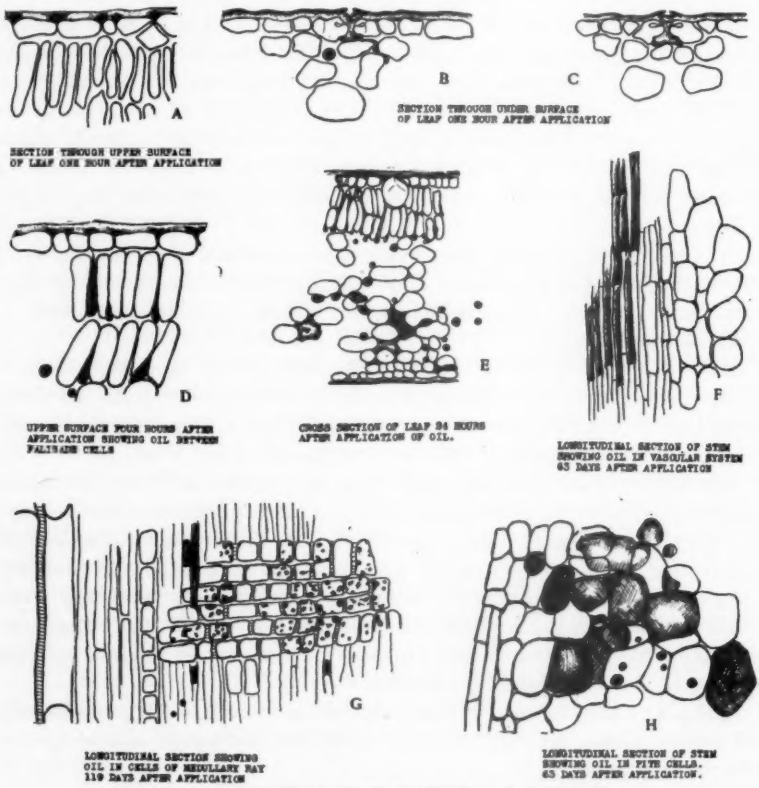
A
B
C
SECTION THROUGH UNDER SURFACE OF LEAF ONE HOUR AFTER APPLICATION
SECTION THROUGH UPPER SURFACE OF LEAF ONE HOUR AFTER APPLICATION
D
E
F
UPPER SURFACE FOUR HOURS AFTER APPLICATION SHOWING OIL BETWEEN PALISADE CELLS
CROSS SECTION OF LEAF 24 HOURS AFTER APPLICATION OF OIL.
LONGITUDINAL SECTION OF STEM SHOWING OIL IN VASCULAR SYSTEM 65 DAYS AFTER APPLICATION
G
H
LONGITUDINAL SECTION SHOWING OIL IN CELLS OF MEDULLARY RAY 110 DAYS AFTER APPLICATION
LONGITUDINAL SECTION OF STEM SHOWING OIL IN PITH CELLS. 65 DAYS AFTER APPLICATION.

FIG. 2. Internal effects of oil applications to leaf surfaces.

epidermal cells and there are a number of small droplets scattered here and there in the intercellular spaces. Little difference is to be observed on the upper surface except that the oil has penetrated deeper into the depressions between the epidermal cells. By the fourth hour, however, oil begins to appear in the palisade parenchyma (fig. 2 D), apparently having been absorbed by the cell walls and carried thence through the epidermal layer. It is first seen as a minute "thread" squeezed between the long cells, gradually expanding as it descends and finally coalescing in drops at the bottom of the palisade cells and passing into the intercellular spaces of the spongy chlorenchyma. At the end of twenty-four hours oil is entering the leaf from both upper and under surface (fig. 2 E). Quantitatively, however, there is little doubt that the greatest penetration occurs beneath. See part III.

As the penetration of oil from the upper surface had not, apparently, been demonstrated previously, this feature was checked with positive results by treating leaves upon the upper surfaces only.

General observations

The oil in the intercellular spaces apparently extracts or dissolves materials from the adjacent cells. In unstained specimens the oil becomes green in color, having evidently dissolved chlorophyll. In stained specimens minute black specks begin to appear in the oil globules at about the third day. These bodies grow larger and coalesce, forming black masses which pass to the periphery of the droplet and become disengaged. At this stage oil can be observed within the cell itself in the form of very minute droplets. It gradually accumulates along the larger veins and passes into the vascular system.

When it became apparent that oil was being translocated steps were taken to ascertain what became of it. Sections were cut from the lateral branch below the treated leaves and also from the main trunk below the point of union with the lateral. Longitudinal and transverse sections of both of these showed large quantities of oil in the phloem (fig. 2 F), the medullary rays (fig. 2 G), and finally in the large storage cells of the pith and old wood fiber of the xylem (fig. 2 H). Some cells became full of oil, others were apparently in process of being filled. Even in those cells already packed with starch grains, oil can distinctly be seen penetrating between them. No oil has been observed in wood of the current season's growth; it is carried across and deposited in the pith parenchyma and the *old* wood fibers. How far down the limb this process extends is not known, but it is probable that most of the oil is deposited in the smaller twigs and limbs and this may account for the great increase in deadwood noted by observers in the field following an application of heavy oil.

Summarizing, the several distinct phases in the elimination of oil from the leaves are seen to be:

(1) Penetration into the intercellular spaces from the leaf surface.
(2) Entrance of the oil into the cell.
(3) Entrance of the oil into the vascular system and translocation down the leaf trace into the stem.
(4) Transfer from the phloem across the medullary rays.
(5) Deposit of the oil in the large storage cells of the pith and in the old wood fibers of the xylem.

Observations on particular oils

Oil number 4.—This was the first one to disappear from the leaves. It is less viscous than oil number 3 although of about the same degree of volatility. It is an unsaturated oil and caused some injury to the leaves, destroying the cells in spots, and causing some of the leaves to drop. The older leaves lost their dark green color and became yellowish, but they regained their color later when the oil disappeared. The last trace of oil was found in the leaf on the 65th day after application. One hundred days after treatment, sections were cut from the limbs below the treated leaves. Both longitudinal and transverse sections showed much oil in the phloem, the medullary rays and the pith and old wood fibers of the xylem.

Oil number 3.—The last trace of this oil was found in the leaf on the 107th day after treatment, 42 days after oil number 4 had disappeared. Oil was still present in the veins of the leaf at this time. One hundred and nineteen days after application and twelve days after the oil had disappeared from the leaf, sections were taken from the stem about seven to eight centimeters below the lowest treated leaf (ten normal untreated leaves occupied this interval). A large quantity of oil was found in the phloem, in the medullary rays and in the pith and old wood fibers of the xylem.

Oil number 2.—The last trace of this oil was found in the leaf 121 days after application. There was still much oil in the veins, and sections from the stem made later showed the same condition as that described above for number 3.

Oil number 1.—This was the most viscous oil tested (106–110 seconds Saybolt). *A large quantity of this oil was still in the leaf 257 days after application.* The translocation of this particular oil is extremely slow, in correlation with its high viscosity. It may, either partially or completely choke certain elements of the vascular system of the leaf for long periods of time. Inasmuch as oil has been found in the leaves of specimens taken in a commercially sprayed grove no less than sixteen months (480 days) after application, it is not improbable that some oil remains in the leaf during most, if not all, of its life period. It was therefore decided to

terminate the test. The stem was sectioned below the treated leaves. As in the previous instances a large quantity of oil was found in the phloem and some in the cells of the pith.

Checks from untreated plots prepared by the same technique were frequently made, both from leaves and stems. Fatty substances, lipoids, resins, essential oils and cutin associated with the leaves and cells were stained but could easily be distinguished from the petroleum by their much darker color.

From the data presented it may be concluded that viscosity is the single factor of greatest importance in determining the mobility of an absorbed oil, and the length of time the oil remains in the leaf and vascular system. The period of persistence is apparently independent of unsaturation provided the tissues are not actually killed. Volatility, as a factor influencing the disappearance of an oil of high boiling point from the intercellular spaces of the leaf, may be disregarded in practise, inasmuch as this study demonstrates that the bulk of this disappearance is due to translocation to other parts of the plant. The lighter oils, being the easiest to move, are the first to disappear.

It appears quite evident that the *accumulation of starch in the leaves, noted in part I of this paper, is due, not to any real stimulation of the plant, but simply to the fact that as the leaf begins to function again, it manufactures carbohydrates which it cannot effectively translocate due to the overloading of the conducting vessels with oil.*

If this condition persists over a protracted period of time it must eventually react unfavorably on the root system, thus weakening the entire tree.

The foregoing data suggest an adequate explanation for the many adverse physiological effects observed in the field following the application of heavy, saturated, white petroleum oil sprays.

Part III

On some factors in the problem of the absorption and translocation of saturated petroleum oil in the living plant

The initial penetration of oil into the leaf.—It is generally assumed that penetration of oil into the leaf is by way of the stomata. On the whole, this belief seems to be sound. The length of time a surface film remains on a treated leaf is found to vary with the type of oil and the character of the cuticle. Thus a light oil (kerosene for example) applied to a succulent, herbaceous leaf which is supplied with large numbers of stomata disappears from the surface rapidly, the time varying from a few minutes to an hour or so. On leaves of the California Live Oak no free oil (of the kerosene type) persists longer than twenty-four hours. No doubt some of

this disappearance is due to volatility. But with a lubricating oil of high viscosity (100–110) the case is different. Oil applied to a *Malva parviflora* leaf did not disappear entirely until the end of the third day. In the case of the leathery evergreen leaves of the California Live Oak (*Quercus agrifolia*) and California Bay (*Umbellularia californica*), the time is very greatly increased. On the Bay leaves there were traces of free oil sixteen days after application, and oil did not disappear from the Live Oak leaves till some time later. The presence of oil was still visible *inside* the leaves of both Bay and Live Oak seven months after application although in apparently reduced amount. Leaves of these two plants were also treated on their upper surfaces only. The period of persistence of free oil was greatly lengthened, and in both leaves when penetration did occur it took place primarily along the margins and tip of the *underside* where the oil had drained from the upper surface. Some dorsal penetration was observed to occur in the Live Oak leaf where it could not be explained by "underrun" but this did not take place to an appreciable extent until the March following the application—a period of three months.

In order to learn more concerning the rate of this initial penetration, leaves were removed from different plants and immersed in pure oil. The time necessary for complete penetration was recorded. The entrance of oil into the intercellular spaces causes the leaf to become translucent. Complete translucence was taken as the criterion of complete penetration. A representative sample of the data obtained is given in the following table (table IV).

The great difference in susceptibility to oil-penetration by different leaves is clearly illustrated in the table mentioned. Even those leaves most resistant to complete penetration (*e.g.*, Live Oak) show considerable and almost immediate partial penetration. Evergreen leaves are, on the whole, more resistant to penetration than herbaceous leaves, and leaves adapted to xerophytic conditions are most resistant of all.

The effect which viscosity plays in penetration is evident in the tests which follow. The leaves of Cranesbill (*Erodium* sp.) immersed in kerosene show complete penetration in 40 to 60 minutes. In saturated petroleum oil of 110 seconds viscosity, it required 300 to 360 minutes.

The resistance of xerophytic leaves of either the succulent or leathery type (*e.g.*, Stonecrop and Live Oak) is due to imperviousness of the epidermis. Penetration in such leaves is very rapid if an artificial opening is made through the epidermis. The succulent tissues of the Stonecrop absorb oil as so much blotting paper if the epidermis be first stripped off. They seem to resist penetration indefinitely if it be left intact. The variation of the method of penetration into different leaves is shown by the following experiment. A lemon leaf and a Live Oak leaf were taken and the tips sliced

TABLE IV

TABLE IV

PENETRATION OF KEROSENE OIL INTO LEAVES OF VARIOUS KINDS. (ISOLATED LEAVES IN PANS OF PURE KEROSENE AT ROOM TEMPERATURE)

SPECIES OF PLANT	TYPE OF LEAF	NATURE AND DEGREE OF PENETRATION AT END OF 15 MINUTES[1]	TYPE OF PENETRATION	TIME OBSERVED FOR COMPLETE PENETRATION TO OCCUR
Montia sp. (Miner's Lettuce)	Succulent, moisture-loving mesophyte	100 per cent.	Uniform	A few seconds; absorbs oil like "blotting paper"
Sambucus sp. (Elder)	Soft; mesophytic. Veins non-sclerenchymatous[3]	About 50 per cent.	Grease-spot[2]	1 hour
Coprosma sp. (New Zealand Looking Glass Plant)	Strongly cutinized arid mesophytic. Midrib and large veins sclerenchymatous	25–30 per cent.	Large checkered[4]	3–4 hours
Rubus sp. (Blackberry)	Soft, hairy, mesophytic. Middle and large veins sclerenchymatous	A few large polygons show partial penetration	Large checkered	10–12 hours
Citrus sp. (Lemon)	Oily, leathery. Non-sclerenchymatously veined	Indefinite blotches show faint partial penetration	Grease-spot	
Umbellularia californica (California Bay tree)	Oily, leathery. With fine network of heavily sclerotic veins	Scattered polygons show faint penetration	Small checkered	About 6 days (140–150 hours)
Quercus agrifolia (California Live Oak)	Leathery, non-oily xerophytic. With fine network of heavily sclerotic veins	Scattered polygons show faint penetration	Small checkered	40 per cent. complete at end end of eighth day (192 hours)
Sedum sp. (Stonecrop)	Xerophytic, succulent (of "cactus type")	None	None	At end of eighth hour there was no indication that penetration had so much as begun. (Not observed further)

[1] Degree of penetration, eye estimates only.

[2] Grease-spot penetration occurs in leaves in which the veins do not form more than a temporary barrier to the lateral diffusion of oil, and in which spread is from a more or less definite center, so that it appears as a circular grease-spot.

[3] The term *sclerenchymatous* is here used in the sense that such tissues are sufficiently abundant around the veins to completely or very nearly stop lateral oil diffusion across the veins.

[4] Checkered penetration occurs where there is a network of sclerenchymatous veins which inhibit lateral diffusion so that each enclosed polygon of the veinous network must be penetrated independently.

off with a razor. The cut leaves were then immersed in oil. The oil rose by capillarity in the lemon leaf and ultimately completely filled the intercellular spaces up to the point of union of the blade with the petiole. In the oak leaf, however, only the mesophyllic sclerenchyma-enclosed polygons which were opened by the razor cut filled with oil. This occurred almost immediately, but there penetration stopped indefinitely.

These observations suffice to illustrate the main features of the phenomena under consideration and to indicate that the factors surrounding oil penetration vary both with respect to the type of oil and the character of the leaf upon which it is applied. The main factor so far as oil itself is concerned is without doubt viscosity.

TISSUE KILLING BY SATURATED PETROLEUM OILS.—When white oils were first used as insecticides, verified evidence as to "burn," *i.e.*, actual tissue killing, was lacking. It has since been established that over-application of some of these oils does result in killing twigs and even branches of orchard trees. There are indications that true leaf-burn may also occur, but in all cases which have been personally observed this occurs only when the intercellular spaces are completely filled with oil and rendered translucent. This does not ordinarily occur in the field because the amount of oil applied is generally less than the capacity of the intercellular spaces.

Leaves of the California Live Oak and California Bay tree were painted with a saturated petroleum oil of 110 seconds viscosity on December 20, 1926. Five months later, of the 57 Bay leaves treated, 3 had dropped; 2 showed a ten per cent. tip burn, and nearly all the rest showed small marginal areas which had been killed by the oil and had eroded away leaving the leaf margins irregular. Of the 33 leaves observed as a check, one had dropped in that period; the rest were normal. The under surface of the Bay leaf normally possesses a distinct waxy bloom. This was destroyed and had not returned at the end of five months.

The results were similar but more severe in the case of the oak leaves. At the end of the five months observation period, of the 48 leaves treated, 36 had droppd; 2 showed 20 per cent. burn and ten appeared nearly normal. No drop or injury showed on the leaves kept under observation as a check. Leaves treated with ordinary kerosene behaved in all respects as the check lot. The bloom on the under surface of the Bay leaves although dissolved as completely by the kerosene as the heavy oil, had returned within six weeks after the application.

On succulent herbs the effect is still more pronounced. Of 100 "Cranesbill" (*Erodium* sp.) leaflets treated with the saturated viscous oil above noted, 33 were "normal" (still impregnated with oil); 27 burned or yellowed and 40 completely dead 15 days after treatment. At the end of one

month all were dead. On this plant kerosene kills or "burns out" large areas of leaf surface where penetration is greatest. This burning occurs within a day or two after the application. But those areas which are not immediately killed do not subsequently die or show other deleterious effects.

TRANSLOCATION OF OIL IN THE LIVING PLANT.—In order to verify the observations reported in part II concerning the translocation of oil from the leaves to the pith of Citrus plants, leaves of the English Laurel (*Laurocerasus laurocerasus*), a glossy leaved evergreen, were painted with saturated petroleum oil of 106–110 seconds viscosity. Six weeks later a section of the stem well below the treated leaves was removed, the bark shaved off, and the resulting woody cylinder carefully scraped to avoid any possible surface contamination; this was finely sliced with a sharp razor and extracted with CCl_4. A similar extraction was made of a sample from a normal branch. From the treated branch 11.2 mg. of CCl_4-soluble material per gram weight of tissue were recovered, while the check yielded just half as much or 5.6 mg. per gram of tissue.

Potato plants were grown and the leaves were treated with the same oil noted above, just prior to the time of tuber formation. After two weeks further growth, the plants were uprooted and the young tubers were washed, sliced and extracted with CCl_4. The tubers of the treated plant yielded 5.4 mg. of CCl_4-soluble material per gram while the check yielded 1.8 mg. The test was repeated, the treated plant yielding 18.6 mg. of CCl_4-soluble material per gram of tuber, the check yielding 7.8 mg. There is a large quantitative discrepancy here which may be explained by the fact that the second lot of material was more finely chopped and was extracted for a longer period of time, but inasmuch as each lot was treated in the same manner as its control, the general conclusion remains unchanged. In addition, the presence of oil exerted a profound effect on tuber formation, those of the treated plant were the size of peas at the time of testing, while those of the check were the size of large marbles, *i.e.*, four or five times as large.

The ability of the plant to absorb and translocate oil was then tested in a third way. Clean river sand was intimately mixed with the same oil noted above in the proportion of 6.8 cc. of oil to 340 grams of sand. Beans were germinated in this substratum and when the plants had reached a height of ten centimeters they were cut off just above the ground line, finely chopped and extracted with CCl_4. From one lot 8.2 mg. of CCl_4-soluble material per gram of tissue were obtained and from a second lot 10 mg. The check yielded 3.8 mg. under the same conditions. The test was repeated with substantially the same results; the plant grown in oiled sand yielded 15 mg. of CCl_4-soluble material per gram of tissue as compared with 5 mg. for the check.

The sand in the preceding experiment did not appear particularly oily and the plants grown therein were apparently normal.

These results demonstrate the reality of cellular penetration of oil and indicate that the plant is capable of translocating oil either from root to leaf or vice versa.

Volatility as a factor in the disappearance of viscous petroleum oil

Volatility has been regarded as *one*, if not the *only*, important factor in oil disappearance from a sprayed plant. As late as August, 1928, de Ong (3) suggests volatility as the primary factor in oil disappearance. This is shown to be in error in the present paper. However, it is certain that even heavy lubricating oils when exposed to air do volatilize to a certain very limited extent. This was tested as follows: Oils were painted in thin films on clean glass plates. One set was exposed to sunlight for a period of eight months under conditions which admitted air but excluded dust. A second set was kept in the dark in the laboratory. Weighings were made from time to time and noted. The results are summarized in table V.

TABLE V

Volatility or weight changes in thin oil films on glass, in free air (November, 1927, to June, 1928)

Oil	Mg. oil to area of glass	Exposure	Per cent. change in weight after 2 months exposure	Per cent. change in weight after 8 months exposure
Saturated White Western Viscosity 106–110	10.8 mg. / 25.5 sq. cm.	Sunlight	Loss 43 per cent.	Loss 89 per cent.
	23.7 mg. / 51.6 sq. cm.	Dark	Loss 14 per cent.	Loss 27 per cent.
Saturated White Western Viscosity 75	16.6 mg. / 51.7 sq. cm.	Sunlight	Loss 87 per cent.	Loss 91 per cent.
	17.0 mg. / 51.7 sq. cm.	Dark	Loss 47 per cent.	Loss 82 per cent.
Saturated White Eastern Viscosity 75–85	15.8 mg. / 51.8 sq. cm.	Sunlight	Loss 12 per cent.	Loss 82 per cent.
	11.0 mg. / 51.2 sq. cm.	Dark	Gain 2 per cent.	Loss 4 per cent.

From these data it is evident that volatility even in the case of heavy oil does occur under the conditions of this experiment. It is important to keep in mind however that similar conditions do not obtain when oil is applied to the plant; in the latter case absorption takes place immediately and is

complete in a few days; once within the tissue, the oil is "bottled up." Optical observations in the latter case render it certain that volatility is negligible and does not nearly equal the figures given above. Furthermore, petroleum oils are not constant boiling point mixtures. Evaporation is therefore most rapid at first and involves only the lighter fractions leaving the heavier and less volatile residue behind. Once inside the leaf, oil disappearance is no doubt due primarily to translocation.

Summary and conclusions

1. Experience has shown that saturated petroleum oils, although not toxic to plants in the ordinary sense of the word, are nevertheless the cause of more or less profound and long continued metabolic disturbances in the plant which may ultimately become seriously deleterious.

2. These metabolic disturbances appear to be due to physical rather than chemical handicaps imposed by the intrusion of the oil into the plant tissue.

3. Oil applied as a free cuticular film, either by means of a brush or a quick-breaking emulsion, persists as such, for a period of a few minutes to fifteen or twenty days, depending primarily upon the morphology of the leaf and the viscosity of the oil.

4. The disappearance of the oil as a free surface film is due to its absorption by the leaf. A certain amount of oil is dissipated in gaseous form at this time. This amount is negligible except in the case of non-lubricating oils.

5. The leaves of plants which are adapted to xerophytic or arid conditions are much more resistant to this initial penetration than softer mesophytic or succulent leaves.

6. The resistance offered to this initial penetration is mostly epidermal, as shown by the fact that penetration in a xerophytic leaf may be extremely rapid once ingress to the interior is obtained. The same factors which protect such leaves from excessive water loss appear to be likewise effective as a protection against the ingress of oil. Penetration is always most rapid in areas rich in stomata but is not necessarily confined thereto. In citrus at least, penetration may occur by seepage between the epidermal cells of the upper (stomata-free) surface of the leaf.

7. Oil penetration is not usually uniform over an entire leaf; certain focal points are first penetrated and the oil tends to spread peripherally from those points. In certain leaves this early peripheral spread is checked more or less completely by the network of veins. Penetration in a leaf of this sort involves the independent absorption by each of the vein-bound polygons of mesophyll.

8. Beginning within a few days after entrance into the intercellular spaces of citrus and extending over a period of many months in the case of

a saturated petroleum oil of 106 seconds viscosity, the oil is taken into the vascular system of the plant and translocated to the storage tissues (pith and xylem parenchyma).

9. In Citrus plants the absorption of oil has been observed directly by microscopical means in the phloem of the leaf traces and stem; in the medullary rays of the stem, and in the pith and xylem parenchyma and sclerenchyma of the stem. No oil has been observed in xylem tissues of the current year's growth.

10. During the period of oil penetration and initial translocation, transpiration is sharply decreased and respiration enormously increased. Photosynthesis becomes temporarily inoperative. This may be due in part to the effects of the oil on the chloroplasts. Intercellular oil is shortly turned green by the extracted chlorophyll.

11. Recovery begins quickest in the case of light oils and is indicated by the return of transpiration and respiration rates towards normal and by the accumulation of carbohydrates in the leaves in abnormally large amounts. This latter phenomenon is correlated with the fact that the conducting vessels (phloem primarily) are still taxed to capacity with the oil and hence cannot adequately handle the carbohydrates now beginning to be produced in excess of the needs of the leaves.

12. The increase of carbohydrates in the leaves of Citrus trees recovering from an oil treatment is therefore in all probability a pathological phenomenon and not an indication of more vigorous synthetic metabolism or "stimulation" as has been maintained.

13. In addition to visual methods (microscopic) the fact of oil accumulation in pith and xylem has been verified by another means. Storage tissues of branches or plants whose leaves had been painted with oil were extracted by means of carbon tetrachloride and the oily materials in such tissues were found to be far in excess of the amounts in similar tissues of untreated plants.

14. It was also found that the plant is capable of transporting oils from the roots upward to the leaves. The leaves of young beans and peas grown in oil-treated sand show a much higher content of oily material than plants grown in normal soils.

15. The intracellular absorption of oils both from the intercellular spaces of the leaf and from the soil indicates the passage of oil molecules through the cell membrane into the cytoplasm of the cell.

16. Microscopic observation of all stages in the translocation of oil was rendered possible by devising a special staining technique which is described in the text.

17. Experiments with oil films on glass plates indicate that volatility is a real factor in the disappearance of even viscous oils in *free air*. In

practise however, due to the enclosure of the oil in the intercellular spaces, it is unquestionably negligible in comparison with translocation.

18. From the effects summarized in the preceding paragraphs the conclusion seems evident that *heavy* white oils (of a viscosity exceeding 60 seconds SAYBOLDT) must be used sparingly and with a great degree of caution, if, in the future, serious ultimate injury is to be avoided.

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OPENING OF STOMATA IN DIFFERENT RANGES OF WAVE LENGTHS OF LIGHT[1]

J. D. SAYRE

(WITH FOUR FIGURES)

The relations existing between the phenomenon of stomatal behavior and the factors influencing this have been widely studied and discussed. There is a great diversity of opinion concerning the significance of many of these factors in regulating stomatal response. This is particularly true for light since this factor may vary greatly in intensity, duration, and quality, depending on the time of year, the degree of cloudiness, the clearness of the atmosphere, altitude, etc.

The author, in a recent paper (**8**) dealing with a study of the stomatal physiology of patience dock, *Rumex patientia* L., included a discussion of the phenomenon accompanying the opening and closing of the stomata of this plant. The variation in the size of the stomatal pore for day and night periods is relatively large in this case.

Historical

Not much work has been reported on the effect of light of various regions of the spectrum on stomata. KOHL (**6**) reported that the rays between B and C and the rays near F were alone effective. FRANCIS DARWIN (**2**) agreed with KOHL as far as the red end of the spectrum was concerned but did not draw definite conclusions about the blue end. LLOYD (**7**) reported normal behavior of the stomata in light between 540 mμ and 700 mμ at the red end and also between 420 mμ and 480 mμ at the blue end of the spectrum. No measure of the intensity of the various regions of the spectrum was given by these investigators. Nor was it stated whether that part of the red beyond 700 mμ was tried.

Methods

Daylight was used as the source of light in these experiments. The plants used, patience dock, were grown out-of-doors. They were covered with tin boxes which were ventilated and light tight. Four ray filters, six and a half inches square, were fitted in the top of each box, the box being sunk several inches in the soil around the plant. Observations of the stomata were made with the leaf in position on the plant. The glass plates were placed over the plants in the evening and observations were made at short intervals during the following day.

[1] Paper from the Department of Botany, The Ohio State University, no. 235.

Two series of ray filters were used. The first series, G38L, G38H, G34, and G24[2], absorb successive portions of the visible spectrum, beginning at the red and extending towards the blue end. The limits of their transmission are shown in figure 1; the shaded areas represent the absorbed regions and the unshaded areas the transmitted regions. This diagram does not

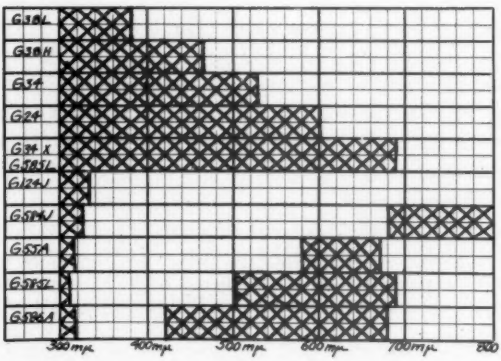

FIG. 1. Limits of transmission of the ray filters used in this work. Shaded areas, absorbed regions; unshaded areas, transmitted regions.

give a true picture of the transmission of all these glass plates. For instances, G55A transmits light to 580 mμ but the percentage transmission between 520 mμ and 580 mμ is very low. For an accurate comparison of the transmission values of these glass plates the Bureau of Standards publications (**1, 3, 4**) should be consulted. The values are also shown in a previous article by SAYRE (**9**), in which the curves of transmission for an equal energy spectrum of all these glass plates are given.

The relative distribution of daylight received upon a horizontal surface was obtained from KIMBALL (**5**). These data which are shown in figures 2, 3 and 4 are the mean values for the different hour angles of the sun, obtained by averaging his values. They are for latitude N 41° and for a cloudless day, September 21.

Kimball's data extend only to 396 mμ in the ultra-violet. Since hardly any light shorter than 300 mμ reaches the earth from the sun, the curve was extended from 396 mμ to 300 mμ as a dotted line. Likewise, in the red end, the last measurements were given at 776 mμ. But as ABBOT and others have shown that the energy from the sun extends far into the infra-red, the curve at this end has been extended as a dotted line to 800

[2] Trade names or numbers of the glass plates used, Corning Glass Works, Corning, N. Y.

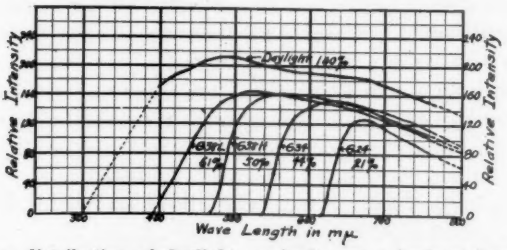

FIG. 2. Relative distribution of daylight received upon a horizontal surface; and the relative and percentage transmission of daylight by the glass plates: series I.

mμ on the same slope as from 700 mμ to 766 mμ. This makes it possible to compare the energy values in this region under the glass plates.

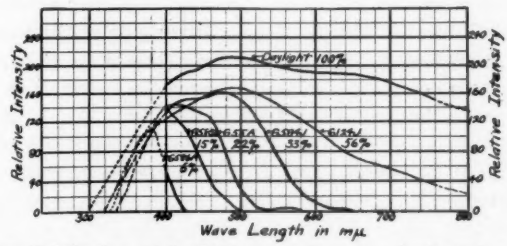

FIG. 3. Relative distribution of daylight received upon a horizontal surface; and the relative and percentage transmission of daylight by the glass plates: series II.

The relative transmission of daylight by the various glass plates was obtained by multiplying their transmission values for an equal energy spectrum by the relative intensities of daylight at various wave-lengths. The percentage transmission, figures 2 and 3, was calculated from the areas under the curves as compared with the curve of daylight.

No instruments were available for measuring the actual intensities of light under the various glass plates, so this method of calculation had to be used. It is recognized that this method may not be very accurate but it is believed that the results obtained allow certain conclusions to be made.

Results

The results of observations made on clear days are considered here, although the results on partly cloudy days did not differ materially from these. Observations of the stomata under the first series of plates, the transmission data of which are given in figure 2, showed that the stomata opened and closed normally. Observations under the second series, transmission data in figure 3, showed the same results, with the exception of

G586A. These observations would indicate that all regions of the spectrum which are not absorbed by these glass plates are equally effective in bringing about the opening and closing of stomata.

Normal opening of the stomata did not occur under G586A, but since the intensity under this plate is very low, it cannot be determined from these results whether intensity or wave-length was the limiting factor. Until more convenient methods of measuring intensities in this region are available and a better source of ultra-violet light is available this part of the problem must be left unsolved.

It is possible by means of a combination of G585L and G34 to make another division of the spectrum at the red end. G585L has a second transmission band in the red. When G34 is placed over it the whole blue end is absorbed and the band in the red from about 690 mμ to far beyond the visible spectrum is left unaffected. The intensity under this combination is rather low because two plates are used and only a limited region of the spectrum is involved. Many observations made under this combination showed that there was no opening of the stomata in this region. They behaved just as in complete darkness. Since the intensity was low it was necessary to prove that this failure to open was not due to lower intensity but to specific wave-length of light. This was attempted by observations of the stomata on a cloudless day. Comparisons were made with similar plants placed under 2–G24 plates, G34 + G585L, in complete darkness, and in full daylight.

Figure 4 shows the relative intensity of the daylight received on a horizontal surface and the relative transmission of 2–G24 and G34 + G585L. These data were obtained in a manner similar to that described for the other glass plates. Table I shows the results of observations on a cloudless

TABLE I

RELATIVE TRANSMISSION OF THE GLASS PLATES AND THE WIDTH OF THE STOMATAL PORE OF PATIENCE DOCK

COMBINATIONS OF GLASS PLATES	LIMITS OF TRANSMISSION	TRANSMISSION OF DAYLIGHT 300–800 mμ	WIDTH OF THE STOMATAL PORE			
			8:30 A. M.	10:30 A. M.	1:00 P. M.	3:00 P. M.
	mμ	*per cent.*	μ	μ	μ	μ
No cover	Full daylight	100	10–12	16–18	0–3[1]	0[1]
Open box	" "	100	10–12	16–18	0–3[1]	0[1]
1–G24	601–Infra red	21	6–8	12–14	18–20	18–20
2–G24	601– " "	12	6–8	12–14	18–20	18–20
G34 + G585L	690– " "	10	1–2	1–2	0	0
Closed	Darkness	0	1–2	0–1	0	0

[1] Leaves badly wilted.

day. The average widths of the stomatal pores are given together with the limits and percentages of transmission of the various glass plates, and suitable checks.

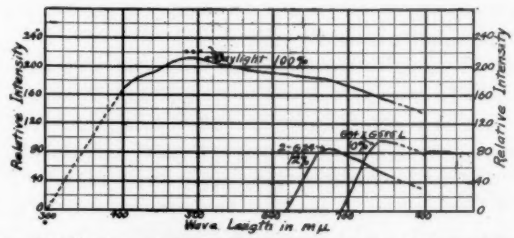

FIG. 4. Relative distribution of daylight received upon a horizontal surface; and the relative and percentage transmission of daylight by 2–G24 and G34 + G585L glass plates.

There was a very slight opening of the stomata under G34 + G585L in the forenoon. Similar behavior was shown in complete darkness. This is a rhythm of the opening of the stomata. It has been reported by several other workers in discussing the behavior of stomata. Some question the existence of such a rhythmic movement, but it can be easily observed and measured in patience dock. It occurs when plants are placed in a dark box after sundown and left there the next day. The stomata remained open under all the other glass plates. Without glass plates they opened normally but closed in the afternoon when the plants wilted badly. Since the intensity under 2–G24 and G34 + G585L was practically the same, the only difference being in the region of the spectrum concerned, we must conclude that wave-lengths of light longer than 680 mμ are not effective in opening of stomata.

In a previous paper (**9**) it was shown that no formation of chlorophyll as indicated by the greening of seedlings occurred in light of wave-lengths longer than 690 mμ. No phototropic response by plants occur in this region of the spectrum, the limit for this being at 526 mμ. Starch accumulation does not occur beyond 690 mμ. This was shown by placing starch-free *Coleus* plants under the glass plates G34 + G585L, and testing them for starch at frequent intervals. The results of this latest investigation show that stomata do not open in light of wave lengths longer than 690 mμ. The conclusion from all of these facts is that the limit of the red end of the spectrum in its effect on plants is at 690 mμ. Wave-lengths of light longer than 690 mμ are probably absorbed by the plant but they apparently produce no chemical or physiological effects. They would be changed to heat energy and raise the temperature of the plant or be lost by radiation.

Conclusions

1. The stomata of *Rumex patientia* do not open in light of wave lengths longer than 690 mμ.

2. The other regions of the visible spectrum (except the violet and ultra-violet) seem to be equally effective.

3. The limit of the effectiveness of the blue end of the spectrum could not be determined from the results of this experiment.

4. It is suggested that red light beyond 690 mμ does not affect plants except as it may be absorbed and changes to heat energy.

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MECHANISM OF CELL WALL FORMATION

O. L. Sponsler

(WITH THREE FIGURES)

As an outgrowth of x-ray studies on the molecular structure of cellulose walls of plant fibers (**12, 13, 14**), several steps in the process by which the cell wall grows in thickness, seem to have become evident. They are associated with the regularity of molecular arrangement in the wall and with the forces which produce that regularity of structure. As the x-ray data, during the progress of the investigations, made it more and more certain that the structural units are glucose residues, and that they are arranged with the regularity of the molecules in a crystal, the concept became clearer and more convincing that the mechanism of cell wall growth in thickness was associated intimately with the forces which are active in causing that regular arrangement of the residues, that is, with forces similar to those which produce crystallization.

The mechanism which is described in the following pages is concerned with only one phase of cell wall growth; that is, with the increase in thickness of the cell wall. While it may be associated also with the increase in surface area as the cell becomes enlarged and even with the formation of the original wall, those two phases are not included in the present discussion. The problem then becomes restricted to a consideration of the mechanism by which new cell wall material is deposited upon older previously formed cell wall.

Layers in cell wall

Where fiber-shaped cells have been studied microscopically (**1, 2, 11, 15**) the wall has been found to consist of many very thin layers (**1, 2, 11**), which seem to indicate a periodicity in the growth in thickness. Each layer is considered as a deposition of new cell wall material upon the surface of an older layer, and while each thin layer may perhaps have two surfaces available for deposition of new material, we are considering here only the innermost layer of the wall and the surface which is in direct contact with the cytoplasm of the cell; that is, the inner surface of the cell wall. Measurements of these layers show that their thickness is equal to several hundred or perhaps several thousand glucose molecules (**12**). The x-ray studies indicate that each of these thin layers of microscopic visibility is made up of still thinner layers which are invisible and which are only one glucose residue in thickness (**12**). The inner surface of the cell wall would be, then, one side of such a layer of glucose residues.

Relation of glucose to cellulose

That the newly deposited material is composed of glucose residues is inferred from the works of various investigators. The new layers have been shown repeatedly through microtechnical work to be composed of cellulose, and cellulose has been shown, through the work of chemists (**7, 4**) to be composed of glucose residues. The residue is a $C_6H_{10}O_5$ type of carbohydrate, an anhydrous form of glucose; but concerning the nature of the material from which the residue is deposited, that is, the material immediately preceding its appearance as cellulose, there is very little discussion in the literature. No experimental evidence is available to show that the anhydrous glucose units of the cell wall are derived directly from glucose molecules. The assumption of its glucose origin is made from the reverse of that reaction; that is, since cellulose may be converted into glucose, the assumption is that glucose in the cell is converted into cellulose. The former is a common laboratory hydrolysis experiment; the latter, glucose to cellulose, a condensation reaction in which water is split off, has never been accomplished *in vitro*. It is not known then whether the glucose molecule, originating through photosynthesis, becomes transformed first to some component part of the protoplasm and then re-transformed into the carbohydrate, cellulose, or whether it is transformed directly into cellulose.

In developing the mechanism of cell wall growth, we are accepting two conclusions which seem to be reasonable: first, that the deposition of new material is made at the interface between the cytoplasm and the wall; and secondly, that glucose is transformed directly into cellulose. While the mechanism is more readily described by accepting these two concepts they might vary considerably without invalidating the mechanism proposed.

Since the process involves a transformation of glucose molecules at the surface of the cell wall into cellulose molecules which become component parts of the wall structure, a detailed picture of the molecular situation at the interface is necessary.

Molecular structure of cell wall

Interpretation of x-ray data shows that the cell wall of plant fibers is constructed of units or building blocks of molecular size which have an orderly arrangement in three dimensions (**6, 10, 5, 12**). The unit of structure proves to be an anhydrous residue of β d-glucose (**13, 9, 7**). The empirical formula for the glucose molecule is $C_6H_{12}O_6$; for the anhydrous residue, $C_6H_{10}O_5$. Spatially these molecules or groups of atoms are three dimensional structures in which the atoms have definite positions allocated to them (**13, 9**). The distances between the atoms, and their location with reference to one another, are known with sufficient definiteness from x-ray

data (**3**) and from chemical experimental data (**4**) to allow a fairly accurate model of the molecule to be constructed. Fig. 2 is a projection of such a model of a β d-glucose molecule (**13, 9**). The carbon atoms are numbered 1 to 6, and the oxygen atoms are indicated by solid black circles. The positions of only two of the 12 hydrogen atoms are shown, as a matter of convenience. These two are represented by the smaller circles attached to the oxygens, forming the OH groups which in turn are attached to the carbons numbered 1 and 4. This glucose molecule becomes an anhydrous residue when the OH is removed from C_4, and the H from C_1.

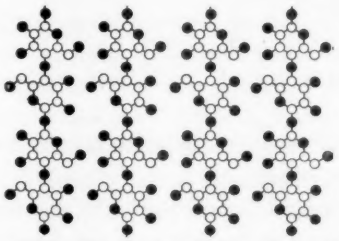

FIG. 1. View of inner surface of cell wall showing four chain molecules. Only four glucose residues of each chain are shown. Solid black circles represent oxygen atoms; light circles, carbon atoms; hydrogen atoms not shown.

In the wall of the fiber each anhydrous residue has a very definite position with reference to the neighboring residues such that the spacing between them in a given direction is uniform. That may be brought out more clearly by referring to fig. 1, where sixteen residues are shown, drawn practically to scale. The distances from center to center of the residues are proportional to the spacings determined from x-ray work with fibers, and their relative positions also are in accord with the x-ray data. They represent, however, a three dimensional structure, and a more truthful picture is obtained when we think of the odd numbered carbons as being raised above the page and the even numbered as being in the plane of the paper. Alternate oxygens are to be considered also as raised above the plane of the paper.

The picture of the structure as given in fig. 1 represents a surface view of the cell wall, such as a minute observer might see, whose senses were attuned to atomic dimensions, if he were inside the fiber looking directly at the inner face of the cell wall. The units or residues would appear to be attached to one another, lengthwise of the fiber, through an oxygen atom acting as a bridge between each two residues. The effect would be that of long chains with the residues acting as links of the chain. The chains themselves, however, because of the greater distance which separates them from one another, would seem to be suspended in space. Fig. 1, then, would

represent a section of the inner surface of the wall showing only parts of four chains, each part containing only four residues.

Inner surface of cell wall

On looking through that surface layer of chains, other similar layers would be seen, all of them parallel to the surface and separated from one another by uniform distances. The distances separating the layers would be nearly the same as those separating the chains. We are especially interested in the layer which forms the inner surface of the cell wall, because it is upon that layer that the new material is deposited. New residues which may be laid down on this surface become arranged in the same orderly manner as the previously deposited residues; that is, they take up positions which result in an extension of the lattice structure towards the interior of the cell.

Inner surface similar to crystal face

The regularity in arrangement of the structural units of the cell wall as revealed by x-ray studies is essentially the regularity of crystal structure; that is, the residues in the cell wall are directly comparable to the molecules in a crystal as, for example, the molecules of sugar in a crystal of sugar. The residues, like the sugar molecules, are not only spaced in an orderly manner but are also oriented in a definite way with respect to one another. It would seem then that forces are involved in locating and orienting the residues in the cell wall, which are comparable to those acting in a similar way on the sugar molecules during the formation of a sugar crystal.

The general conception concerning these forces is that they are due to the electrical conditions occurring in the component atoms, which set up electrostatic force fields around the molecule; and that these force fields are responsible for the cohesion between the molecules in the crystal (**8**). In some way these force fields produce the orientation of the molecules when they are being deposited on the crystal face; and they also determine the location of the molecule. The position which the molecule is forced to take is one of minimum potential energy which is also the position of a unit of the crystal lattice.

Since the glucose residues act as lattice units of the cell wall, one is led to think that the force fields which determine their position and orientation are similar to those acting when molecules are deposited on a crystal face. The inner surface of the cell wall then may be considered as similar to a crystal face as regards the distribution of forces which are involved in the deposition of new lattice units.

Glucose molecule and its anhydrous residue

There is, however, this difference, that the sugar molecule has very probably the same structure in solution as it has after it is deposited as a unit of the sugar crystal; while the glucose molecule in the cytoplasm is slightly different in its structure from the residue which acts as the unit in the cell wall. As a matter of fact there is a great probability that the β d-glucose molecule is only very slightly different from its anhydrous residue, the difference between them being the loss of one OH group and one H atom from carbons number 1 and 4, respectively, or vice versa. That may be visualized readily from fig. 2. It may be that the possibility of the formation of cellulose is due to that particular difference and similarity. There seems to be no fundamental reason for thinking that the component atoms of the molecule and of the residue differ in their general spatial relations to one another, although that has not been definitely proved.

FIG. 2. A single glucose molecule. The carbon atoms are designated by number. Only two hydrogen atoms are shown.

Transformation of glucose to cellulose

When we accept the general similarity of the two, and assume that the glucose molecule could be deposited on the surface of the cell wall as though it were a residue, a very interesting state of affairs is brought out. The molecule would probably take a position of minimum potential energy, which would be the position of a unit of the lattice; also it would be oriented nearly, if not exactly, as a residue would be if in that place. That may become clearer by imagining a glucose molecule (fig. 2) as finding the proper position over a surface such as represented by fig. 1, and thinking of the molecule as the starting point of a new layer. A second molecule may be expected to fit into an adjacent position. This could be represented by substituting fig. 3 for fig. 2. The force fields, or we may say the cohesion, should hold these two molecules practically as firmly to the surface layer as would be the case if they were anhydrous residues. When the molecules of fig. 3 are forced into the proper orientation by forces of the surface layer, the two OH's attached to the numbers 1 carbons are brought into close proximity. That is decidedly an advantageous spatial relation for a condensation reaction to take place. The result of such a reaction is the formation of a water molecule, $OH + H = H_2O$; and at the same time, the formation of an oxygen bridge between the two glucose

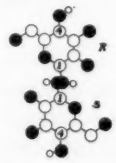

FIG. 3. Two glucose molecules placed as they are oriented in the cellulose chain, to show the position of the OH's at time of condensation. Smaller circles represent hydrogen atoms.

residues in which the remaining O is used. The paragraph may be summed up by saying that the force fields, resulting from the regular distribution of the glucose residues in the wall, have made the conditions favorable for a condensation reaction, such as the organic chemist usually expects when two OH groups are brought close together.

Relation of lattice to long chain formation

The conditions are made favorable, however, for the reaction to take place in one direction only. It may be seen in fig. 1 that the distance from center to center of the residues is too great laterally for condensation to occur between adjacent units of two chains; while in a lengthwise direction of the chains the distance is extremely favorable for the reaction. That would allow an indefinite number of glucose molecules to be brought into position in which the condensations would be made probable; and the formation of a long straight chain of residues linked by oxygen bridges might readily follow.

Relation of glucose structure to chain molecule

The structure of the β d-glucose molecule also favors the straight chain formation. Evidence from both chemical (**4**) and x-ray work (**13, 9**) shows that the oxygen bridges are associated with carbons number 1 and 4. These two carbon atoms are found to be located on opposite sides of the glucose molecule model. That location places them in positions in the molecule which are decidedly favorable for the condensation reaction, and in positions which permit of a straight chain formation.

Evidence of a third factor

Although the molecular and atomic arrangements and dimensions all tend towards the formation of cellulose chain molecules, the mechanism described above cannot account for all of the observed facts. The mechanism mentioned requires only two factors, (1) the cellulose surface and (2) the presence of glucose molecules. It is well known, however, that glucose may be present in a cell and no growth take place in the thickness of the wall. A third factor, then, must be involved in the growth. It is also well known that no growth in thickness of the cell wall occurs after the protoplasm has become inactive, although glucose may be present. The third factor, then, must be associated with some reaction which can occur only when living protoplasm is present. Another well known fact, that the wall may grow on one side of the cell and not on another side, indicate that the third factor is localized in the cell. And since chunks of cellulose do not occur at random throughout the protoplasmic mass inside the cell it would seem that the third factor is localized only at the interface between the cytoplasm and the wall.

Suggestions as to the nature of that third factor are rather meager. Speculation suggests many lines of approach. Careful studies of the conditions inside of the cell at the time the cell wall is being thickened, studies with a view point to determining the concentration of glucoses, and the forms in which they occur, the rate of respiration, the water content of the cytoplasm, the presence of dehydrating bodies, etc., may throw some light upon the remaining parts of the mechanism.

The use of the expression "third factor" or "third body" is, of course, merely symbolical of whatever process or processes must be carried on to complete the condensation reaction which was made possible by the surface forces of the inner face of the cell wall.

Summary

When considering the growth of a cell wall, three stages in the process may in general be recognized: (1) the formation of the original or first layers of cellulose; (2) the surface area growth of the wall as the cell becomes larger; and (3) the increase in thickness of the wall. The third stage only is under consideration in this paper. An attempt has been made here to show that inherent in the molecular structure of the wall are forces which must share very largely in the process of building up the wall itself in thickness at least.

An outstanding feature in the mechanism of thickness growth, it may be pointed out, is the part played by a *surface of regularly spaced molecular units.* The necessity of a surface of some kind on which cell wall material may be deposited is in accord with microscopic studies; but concerning the molecular structure of the surface, only one kind of foundation surface, that of the cellulose wall itself, has so far been investigated. The preceding discussions, then, refer only to cellulose surfaces in which the regularity of arrangement of the structural units, β d-glucose residues, suggests a comparison with crystal surfaces in which large molecules act as structural units. From that comparison one is led to conclude that the forces of crystallization form a part of the mechanism by which glucose molecules become converted into cellulose. Upon following that line of thought farther, it was found that on account of the particular structure of the glucose molecule an opportunity was provided for a condensation reaction which would produce long straight chains of residues, or cellulose molecules.

It was pointed out that crystallization forces alone could not account completely for the transformation of glucose to cellulose; that a third factor which seemed to be associated with living protoplasm was necessary. No discussion of the nature of that third factor is given.

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THE PURE PIGMENTS, CAROTIN AND XANTHOPHYLL, AND THE TSWETT ADSORPTION METHOD[1]

F. M. SCHERTZ

Introduction

Ever since the yellow plastid pigments have been investigated in plant organs, especially in leaves, there has been considerable controversy regarding the nature and the number of the pigments present. STOKES supposed that there were two and SORBY three xanthophylls. BORODIN observed that many crystallizable yellow substances, whose solubilities differed, were present in leaves. He recognized two groups, the carotins, to which belong crystals easily soluble in benzene and difficultly soluble in alcohol, and a second group the representatives of which dissolve very slightly in benzene and easily in alcohol. ARNAUD believed that only a single yellow pigment was present and that this was carotin. MONTEVERDE, TSCHIRCH, TSWETT and SCHUNCK confirmed and enlarged upon these observations. Later WILLSTÄTTER and MIEG isolated a representative of each of the groups as designated by BORODIN. The large yield of carotinoids obtained by WILLSTÄTTER and his coworkers makes it seem highly improbable that other yellow plastid pigments are present (**10**). A very complete review of the literature is given in the monograph of WILLSTÄTTER and STOLL in chapter XII.

TSWETT by means of his chromatographic adsorption apparatus has attempted to further resolve the yellow pigments. For example, by filtering a carbon disulphide solution of the leaf pigments through a column of calcium carbonate, carotin passes through without being adsorbed. In addition to the green pigmented zones are four zones of yellow pigments, which TSWETT distinguished as α, α^{I}, α^{II}, and β. In the spectrum they show small relative differences in the position of their absorption bands, and as yet these pigments have not been crystallized or isolated in the pure state. TSWETT holds the view that the xanthophyll of WILLSTÄTTER and his coworkers is a mixture of two or three isomorphic xanthophylls while WILLSTÄTTER has suggested that it is possible that the original pigments have been changed by oxidation during the process of separation. It is the purpose of this paper to support the contention of WILLSTÄTTER.

[1] Soil Fertility Investigations, United States Department of Agriculture, Washington, D. C.

Inconsistencies in recent literature

Results described in recent literature show that the method of TSWETT has been employed, and in a paper on xanthophyll (**5**) certain inconsistencies reported in the literature have been noted by the writer. The inconsistencies referred to are to be found in a paper by GILL (**1**) and others in a paper by PALMER and ECKLES (**3**).

GILL states that "in certain cases none of the pigments is extracted from a petroleum ether solution of the pigments by 80–90 per cent. alcohol, while large amounts of the pigments are adsorbed by calcium carbonate." Since the pigment is not extracted from petroleum ether it would be carotin; and since it is adsorbed by calcium carbonate it would be xanthophyll. But surely it cannot be both pigments at the same time.

PALMER and ECKLES report that the "xanthophyll-like constituents of yellow corn were not adsorbed by calcium carbonate from a carbon disulphide solution and on the other hand the pigments could be completely extracted from a petroleum ether solution by 80 per cent. alcohol."

No attempt was made by these authors to explain their results and in fact nothing was said in their work regarding the peculiar behavior of the yellow pigments, with which they were dealing. It would appear then that either the method of fractionation as described by WILLSTÄTTER must be faulty or that TSWETT's adsorption method does not separate xanthophyll from carotin.

Before turning our attention to an explanation of the inconsistencies mentioned above it will perhaps be of interest to have briefly summarized some of the statements made by various writers concerning the different xanthophylls which have been described.

THE XANTHOPHYLLS OF KOHL

KOHL (**2**) described two xanthophylls. The xanthophyll as described by SCHUNCK, KOHL called α xanthophyll, which has four absorption bands beyond the Fraunhofer line F. This xanthophyll was not obtained in crystalline form, yet it has many of the properties of the xanthophyll described by WILLSTÄTTER and may be regarded as representative of the xanthophyll which we know today.

The xanthophyll described by TSCHIRCH, KOHL has designated as β xanthophyll. This xanthophyll KOHL states is present in all types of leaves, green, yellow and autumnal; and a solution of it possesses only end absorption. β xanthophyll was present mostly in the cell sap and could be extracted by boiling water, forming a dark yellow, yellow, or yellow brown solution. When ammonia was added the aqueous solution darkened and with alkali it became orange colored. Petroleum ether would not extract

any of this yellow pigment from an alkaline solution. If the leaves were extracted with alcohol after the water extraction, and ether and water then added, the ether took up the carotin and traces of α xanthophyll, while some of the α xanthophyll remained dissolved in the aqueous alcohol. Normal chloroplasts were found by KOHL to contain little α xanthophyll and little β xanthophyll while autumn leaves contained little α xanthophyll and much β xanthophyll.

Now, practically every word of the above description of the two xanthophylls is applicable to pigments which are well known today. One of these pigments, α xanthophyll, is now called xanthophyll while the data above for β xanthophyll exactly describes what are known today as the flavones. It is apparent then that no further discussion is needed regarding the two xanthophylls, α and β, described by KOHL.

THE XANTHOPHYLLS OF SCHUNCK

In a paper by SCHUNCK (**6**) it is reported that two yellow coloring matters were found accompanying chlorophyll in crude alcoholic extracts of healthy green leaves. The one was called chrysophyll (carotin) and the other xanthophyll (a pigment not readily adsorbed by charcoal). A third yellow pigment was sometimes found along with the xanthophyll. This pigment gave no absorption bands, but only an obscuration in the violet and ultra-violet region of the spectrum. In the latter case separation could be effected by ether. Evidence showed that other yellow coloring matters existed. Xanthophyll was believed by him to be the predominating yellow coloring matter accompanying chlorophyll in the healthy green leaf. Chrysophyll (carotin) and xanthophyll each gave characteristic absorption bands in the violet and the ultra-violet region, the former consisting of three bands and the latter of four, but in slightly different positions.

It is certain that SCHUNCK was dealing here with carotin, xanthophyll and the flavones as we know them. In addition, he seems to have been dealing with other yellow pigments, probably oxidation products of carotin and xanthophyll.

In another paper by SCHUNCK (**7**) three xanthophylls, L, B, and Y, are described. Alkalies appeared to have no effect upon the members of this group and saponification did not appear to alter them. They were never obtained in the crystalline form but each was found to possess three absorption bands. These pigments were distinguished by different spectroscopical reactions with acids. Hydrochloric acid had no immediate effect upon the spectral absorption bands of L xanthophyll, but produced an immediate and striking effect upon the spectra of B and Y xanthophylls, their solutions becoming paler in color. The solutions then became greenish blue and

finally colorless. L xanthophyll was more soluble in carbon disulphide than the B and Y forms.

Summarizing the properties of L xanthophyll as given by SCHUNCK may assist us in ascertaining the true nature of the pigment. Hydrochloric acid had no immediate effect upon L xanthophyll, but the bands faded after a time. Nitric acid caused the color of the pigment to fade at once. The L xanthophyll was more soluble in carbon disulphide than were the B and Y forms. The L form was more or less stable, its alcoholic solution showing but little change even after several weeks when kept away from the light. With hydrochloric acid no color reaction was produced in either alcoholic solutions of chrysophyll or those of L xanthophyll. Towards acids, hydrogen peroxide and nascent hydrogen, chrysophyll and L xanthophyll behaved very similarly although chrysophyll withstood the action of hydrochloric acid to a greater extent. The absorption bands of the two are very similar, a slight shift being the only difference.

All of the properties reported for L xanthophyll, in the above paragraph, are in perfect accord with those for carotin and there is no doubt that the L xanthophyll described was carotin. The only reason why SCHUNCK did not call the two pigments (L xanthophyll and chrysophyll) identical appears to be due to the fact that L xanthophyll was never obtained in the crystalline state. He remarks that "in none of the flowers experimented with was any chrysophyll obtained." Today we know that it is highly probable that carotin is present in all of the twenty flowers with which he worked, since the experience of the writer has shown that carotin often may be present in plant materials but may not always be obtainable in the crystalline form.

Our attention will now be directed to his B and Y xanthophylls. SCHUNCK found that B xanthophyll greatly exceeded Y in the tissues examined. The bands of the xanthophylls exhibited a gradual shifting toward the violet, those of chrysophyll being the least refrangible, the order being chrysophyll, L, B, and Y xanthophyll. On standing, the three xanthophyll extracts generally gave an indication of a fourth more refrangible band. Practically the only distinction SCHUNCK made in his B and Y xanthophylls was the slight difference in the position of the absorption bands. He always speaks of B and Y xanthophyll having certain properties and these properties in general agree very closely with those for xanthophyll. Also, he does not mention having found B and Y in the flowers of the same plant, all of which tends to show that the two are the same. The evidence then seems to show that SCHUNCK was working with carotin and xanthophyll, the L xanthophyll being carotin and the B and Y forms being xanthophyll. Oxidation of the pigments, too, must have played a part in his observations (**8**).

THE XANTHOPHYLLS OF TSWETT

TSWETT (**9**) apparently deals with several different xanthophylls. To obtain a clear idea of his work it will perhaps be best to start with the extraction of the pigments from the leaves according to his procedure (**8**, p. 321). Fresh leaves, preferably *Lamium*, were ground with fine emery in a mortar. Extraction was accomplished with petroleum ether which contained 10 per cent. alcohol. The green solution was then washed repeatedly in a separatory funnel with double its volume of water. After the turbid solution was filtered or centrifuged it was suitable for adsorption experiments. If the petroleum ether chlorophyll solution was shaken with precipitated calcium carbonate the pigments were precipitated, and with an excess of the precipitating agent only carotin remained in solution. If the carotin solution was shaken with 80 per cent. alcohol, the aqueous alcoholic phase remained colorless. The pigments were then completely removed from the precipitating agent by means of alcohol containing petroleum ether. After treating the bluish-green solution with 80 per cent. alcohol, the bluish tinged petroleum ether solution contained the chlorophyll while the alcoholic layer contained chiefly the xanthophyll.

If to the original petroleum ether solution of chlorophyll the adsorption agent was added gradually till the fluorescence disappeared, then the xanthophyll as well as the carotin remained in solution. The xanthophyll was freed of carotin, by treating the carotin-xanthophyll solution with an excess of the adsorption agent. The pigment xanthophyll was extracted from the adsorption medium by means of alcoholic petroleum ether. The xanthophyll mixture thus obtained showed absorption at 480–470 mμ and 452–440 mμ. If shaken with 80 per cent. alcohol the pigment remained almost completely in the alcoholic phase.

From the above description there is no doubt that TSWETT was really dealing with carotin and xanthophyll, for the solubility in ethyl alcohol and the absorption in the violet region both indicate xanthophyll; and the manner in which the carotin solution was obtained shows us clearly that his pigments were carotin and xanthophyll as we know them today from WILLSTÄTTER'S work. One point may well be taken up here; from the foregoing description of the method of preparing the pigments for use in the adsorption work it is very evident that TSWETT was never at any time dealing with pure pigments for not once were the substances crystallized, nor did he report any attempt made at crystallization. It may be assumed then that each of the solutions (carotin and xanthophyll) contained considerable traces of the other yellow pigment as well as large amounts of oxidation products of both of the pigments. This point must be clearly evident to any one who has isolated crystals of carotin or xanthophyll from their

respective carotin (petroleum ether) and xanthophyll (alcohol) fractions, and has noticed the large amount of noncrystallizable material remaining.

The chlorophyll mixtures used by TSWETT in his adsorption apparatus were generally obtained as described below: The leaves were ground with fine emery and a little calcium carbonate or magnesium oxide as neutralizing material, and were then extracted by means of alcoholic petroleum ether (1:10), after which the alcohol was carefully washed out of the petroleum ether by means of distilled water. The washing here was very thoroughly done, otherwise the alcohol and the water interfered with the adsorption bands of the chromatogram. Often the neutralized ground leaves were extracted with alcohol, acetone, ether or chloroform, which was distilled off in a vacuum and then carbon disulphide was used to again dissolve the pigments. The carbon disulphide solution of the pigments was used to obtain the chromatogram.

TSWETT recognized seven zones in the chromatogram and described them as follows, beginning at the top:

Zone I—Colorless.

II—Yellow, xanthophyll β, an alcoholic solution of the pigment absorbed in the regions 475–462 mμ and 445–430 mμ. The pigment remained in the alcoholic phase of an aqueous alcoholic, petroleum ether system. Hydrochloric acid turned the alcoholic solution blue. All of the pigments of the various zones may be washed out quite readily. Ten per cent. alcoholic petroleum ether washed out xanthophyll from the adsorption medium.

III—Chlorophyll β.

IV—Chlorophyll α.

V—Yellow. Xanthophyll α^{I} and α^{II}. If benzene is poured into the tube containing the zones of color, zone V washes out slowly and forms a double ring.

Accordingly TSWETT assumed that two xanthophylls were present. The absorption spectrum of zone V was pushed a little further toward the ultraviolet than was the spectrum of xanthophyll α.

VI—Colorless.

VII—Orange yellow. Xanthophyll α. This pigment remained in the alcoholic phase of an aqueous alcoholic petroleum ether mixture. It bleached when hydrochloric acid was added and did not become blue. The position of the bands was 485–470 mμ and 455–440 mμ in an alcoholic or petroleum ether solution. When benzene was poured into the tube, zone VII was washed out at once.

Carotin passed through without being adsorbed. The carotin remained in the petroleum ether layer of an aqueous alcoholic petroleum ether mixture. The addition of concentrated hydrochloric acid did not turn its alcoholic solution blue. The absorption of the carbon disulphide solution was 525–510 mμ, 490–472 mμ and weakly at 460–455 mμ. In petroleum ether the bands were located at 492–475 mμ and 460–455 mμ.

Only the portion of TSWETT's work which refers to the yellow pigments has been quoted in full for it is with the yellow pigments alone that this paper is concerned. We are concerned not with the carotin fraction but with the various xanthophylls which he has described. It will be observed that impure solutions of pigments were used in all of the work. Large amounts of oxidized products of both carotin and xanthophyll undoubtedly were present in the pigment mixtures used.

THE WORK OF PALMER AND ECKLES AND TSWETT'S CHROMATOGRAM

Perhaps PALMER and ECKLES (**4**) have done more with TSWETT's absorption column and his methods than any other worker or group of workers. The yellow pigments were separated from the butter fat by saponifying for ½ hour with a 20 per cent. solution of alcoholic potash, at the temperature of the boiling solution. Three volumes of distilled water were added and the pigment was shaken out with ether, which was then washed and dried. The plant pigments of alfalfa were extracted by adding carbon disulphide to the ground material and allowing it to stand several days.

In all of the experiments on butter fat and alfalfa it is noteworthy that a carotin-like substance passed through the calcium carbonate in the chromatogram. A xanthophyll-like substance was adsorbed somewhat and then there was a yellowish substance which could not be removed at all by carbon disulphide, but could be removed by alcoholic petroleum ether from the chromatogram. In the case of butter fat (**3**, p. 352) the portion which passed through constituted about 98 per cent. of the total pigment. The adsorbed portion consisted of approximately 2 per cent., while about 8 per cent. of the portion which passed through was alcohol soluble. The authors do not seem to make any distinction as to whether the pigments are fractionated by dissolving them in a petroleum ether solution and then fractionating by means of alcohol, or by dissolving them in alcohol and then fractionating by adding successive portions of petroleum ether. The manner of fractionating would make a great difference in the quantitative results, for by extracting the alcoholic solution, the xanthophyll fraction would appear to be less than it really is, and, besides, part of the xanthophyll would appear to be carotin. (The proper method of separating carotin from xanthophyll is to fractionate a petroleum ether solution of the mixed pigments repeatedly with aqueous methyl alcohol.)

PALMER and ECKLES in their feeding experiments to determine the pigments present in butter fat, studied the pigment content of the feeds used. In the unsaponifiable pigments (**3,** p. 361) of cottonseed meal they found equal parts of carotin and xanthophyll, the xanthophyll being made up of at least five different constituents according to their adsorption properties. The chief xanthophyll is not adsorbed to any extent by calcium carbonate from its carbon disulphide solution, which also shows in the spectroscope a shifting of the bands toward the blue, from the normal xanthophyll bands. The remainder of the xanthophylls were held so firmly by the calcium carbonate that they could not be readily removed by a stream of carbon disulphide. One per cent. alcoholic petroleum ether would easily wash all of them out.

The yellow pigments of yellow corn (**3,** p. 362) were not adsorbed from either petroleum ether or carbon disulphide by calcium carbonate. Petroleum ether readily extracted the pigment from its concentrated 80 per cent. alcoholic solution but it could be completely reextracted from its petroleum ether solution by fresh 80 per cent. alcohol. In this respect it differed from any xanthophyll-like pigment yet investigated. The minor constituents of the corn pigments, as to solubility, spectroscopic, and adsorption properties resemble carotin. A carbon disulphide solution of yellow pigments from carrots was passed through the chromatogram. Only a small portion of the pigment was adsorbed and this was not differentiated into zones. It was readily washed out with alcoholic petroleum ether. The xanthophyll here was only 3–4 per cent. of the entire yellow pigments. Zone I was entirely different from all of the others; its alcoholic solution turned bluish-green when concentrated hydrochloric acid was added. This xanthophyll the authors believed to be identical with xanthophyll β of TSWETT and xanthophyll β of SCHUNCK.

Since the authors usually extracted the yellow pigments from alcohol by means of petroleum ether, not much reliance can be placed upon their quantitative data regarding the amount of carotin or xanthophyll present in the various substances.

All of the xanthophylls reported in this paper have already been correlated except those described by TSWETT, GILL, and PALMER and ECKLES and these will now be considered.

EXPERIMENTS WITH PURE XANTHOPHYLL AND PURE CAROTIN

Investigation showed that by using practically pure solutions (**5**) of xanthophyll only a very little, or none, of the pigment was absorbed when a carbon disulphide solution of xanthophyll was passed through a tube filled with calcium carbonate. If the carbon disulphide solution after being passed through a calcium carbonate column was allowed to stand for a day or more,

and then again put through the tube, more of the pigment was adsorbed. Usually the longer the carbon disulphide solution of xanthophyll stood, exposed to air and sunlight, the greater would be the amount of the adsorbed yellow pigment. This adsorbed yellow pigment, when washed out by means of 95 per cent. ethyl alcohol, and taken up in petroleum ether, may be wholly extracted from the petroleum ether by means of 92 per cent. methyl alcohol; hence it had the properties of xanthophyll.

Carotin behaved in approximately the same way that xanthophyll did. Pure solutions of both in carbon disulphide left only traces of yellow pigment in the adsorption column, while solutions which were partially oxidized left a band of yellow in the calcium carbonate tubes. Xanthophyll always left much more of the adsorbed pigment behind.

An experiment may serve to show the relative properties of the two pigments. After adding pure solvent (CS_2) to an adsorption tube, 5 cc. of pure carotin solution was added. In about 4 minutes, and by adding 10 cc. of pure carbon disulphide, all of the carotin had passed through the column, save a mere trace. An equal amount of xanthophyll solution of approximately the same concentration as the carotin solution required more than 15 minutes and at least 50 cc. of carbon disulphide before the carbon disulphide passing through the adsorption tube became colorless. A yellow zone usually remained. This indicated in a way the relative speed with which the two pigments passed through a calcium carbonate column. It is certain that carotin passed through the column much more rapidly than xanthophyll did, but in a mixture of the two pigments it would be very difficult to say just when carotin has ceased coming through and when xanthophyll had begun. Or perhaps the period of the last traces of carotin and the first traces of xanthophyll might easily overlap each other and thus there would be no sharp separation of the two pigments by the adsorption method. The method cannot be recommended to distinguish carotin and xanthophyll. However, it might be of some value after considerable experience with it. On the other hand, the method of separating the two pigments by means of petroleum ether and methyl alcohol is absolutely reliable, easy to perform, even by beginners, and in every way is far more satisfactory than the adsorption method. There is little chance of making errors by this method.

Explanation of the inconsistencies in recent literature

There remains for us to make clear a few of the cases which have been cited above. In considering the regions or zones described by Tswett, it is necessary to use caution for it is to be remembered that chlorophyll in any one of its many forms may appear in the different zones and cause color changes which are very misleading.

Zone II (xanthophyll β) of TSWETT is undoubtedly composed of oxidized xanthophyll, since pure xanthophyll would wash out by further washing with carbon disulphide. Zone V (xanthophyll α^{I} and α^{II}) and VII (xanthophyll α) are apparently made up of pure or partially oxidized xanthophyll, but an explanation of why it separates into zones here, is another matter. Since stages in the oxidation of xanthophyll have not been studied, nor the oxidation of xanthophyll ever controlled, it is not possible to say definitely that these zones are stages in the oxidation of xanthophyll; but the fact that the xanthophyll separates into zones and that oxidation of xanthophyll causes a band to be found in the adsorption column, all lead us to suspect that the phenomenon of TSWETT is probably the result of different stages of oxidation of xanthophyll.

The yellowish substance, which could not be washed from the chromatogram by carbon disulphide and which PALMER and ECKLES found in butter fat, is undoubtedly an oxidized carotinoid. The chief pigment of cottonseed meal was found to be xanthophyll (by PALMER and ECKLES) which was not adsorbed by calcium carbonate. This non-adsorption is not surprising, for pure xanthophyll is not adsorbed. The yellow corn pigment was not adsorbed by calcium carbonate, and petroleum ether extracted the pigment from 80 per cent. alcohol; but the pigment in turn could be completely re-extracted from the petroleum ether by means of 80 per cent. alcohol. According to the authors it differed from all other xanthophylls in this respect. Since all of the above characteristics are those of pure xanthophyll, nothing is strange about the behavior of the pigment. In the case of the carotinoids from carrots only a small portion of the pigment is adsorbed, which was exactly what would have been expected with carotin.

The statement by GILL is perhaps the most difficult to harmonize with the properties of the yellow pigments. He states that in certain cases (yellow corn, mustard, and orange peel) none of the pigments is extracted by 80 per cent. alcohol from petroleum ether. This would indicate carotin in all 3 plant materials. Further he says that large amounts are adsorbed by calcium carbonate; this would indicate xanthophyll. Since oxidized xanthophyll behaves in regard to alcohol and petroleum ether in the same manner as pure xanthophyll, the adsorbed portion could not have been xanthophyll nor its oxidation product. The only other possibility is that it is oxidized carotin, since pure carotin would not be adsorbed, and this is not possible in the case of the pigment of yellow corn, which was one of the cases in question; the pigment present in yellow corn is known to be xanthophyll with only a trace of carotin. Since PALMER and ECKLES give exactly the opposite properties regarding the adsorption of the chief pigment of yellow corn and since the properties of this pigment as they describe it agrees with

those of xanthophyll it is assumed that the observations of PALMER and ECKLES are correct.

The pigment extracted from orange peel has been investigated by the writer and it was found that none of the pigment was adsorbed from a carbon disulphide solution by calcium carbonate, while none or only a very little was extracted from petroleum ether by 80–92 per cent. methyl alcohol. This would indicate that the greater portion or nearly all of the pigment in orange peel is carotin. Here again it is shown that adsorption of the pigment is questioned, for the observations reported here, and those of GILL who used TSWETT's methods are contradictory. However, observations made in this investigation and those of PALMER and ECKLES, regarding the distribution of the pigment between petroleum ether and methyl alcohol agree. These facts show again the unreliability of the method described by TSWETT for distinguishing carotin and xanthophyll, and also that the methods of WILLSTÄTTER may be relied upon.

Summary

1. A brief summary is given of the work on the yellow pigments.

2. Certain apparent inconsistencies in the literature are pointed out and published statements regarding the carotinoids are shown to harmonize with known data for xanthophyll and carotin.

3. The xanthophylls (α and β) of KOHL are shown to be xanthophyll and flavone respectively.

4. The chrysophyll, xanthophyll, and the third yellow pigment of SCHUNCK are shown to be carotin, xanthophyll, and flavone respectively. SCHUNCK's L xanthophyll apparently is carotin, while his B and Y xanthophylls are xanthophyll.

5. TSWETT's xanthophyll β apparently consists of oxidized xanthophyll. His xanthophylls α, α^{I} and α^{II}, from his descriptions, have properties very similar to those for pure xanthophyll, and most probably result from slight oxidation of the pure pigment.

6. Pure carotin has been shown to be unadsorbed by calcium carbonate. Pure xanthophyll is only relatively adsorbed, that is, it passes through an adsorption column more slowly than does carotin.

While TSWETT's methods have been shown to be unreliable in identifying and distinguishing carotin and xanthophyll, the methods of WILLSTÄTTER have been shown to be more dependable for this purpose.

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GROWTH STUDIES ON FRUITS. RESPIRATION OF TOMATO FRUITS[1]

FELIX G. GUSTAFSON

(WITH THREE FIGURES)

The measurements of respiration here reported have been made as a part of a general study of the growth of tomato fruits. The fruits used have partly come from plants grown in the open during the regular growing season and partly from plants grown in the greenhouse during the winter. The plants grown in the greenhouse have been supplied with additional light during the period of shortest days. The fruits from these plants have been normal in all respects so that comparisons can be made between fruits grown out of doors and indoors.

The experiments have been conducted and the amount of carbon dioxide determined as reported by HOVER and GUSTAFSON in another paper (**6**), except that the length of time of each experiment has been much shorter. In all of the experiments cited only one fruit was used in each container, so that the figures given are not averages for several fruits, but rather values for individual fruits. In all of these experiments the fruits were picked from the vines about a half hour to an hour before the experiment was started.

The first experiments were conducted in the fall of 1927 on Livingston Globe and John Baer tomatoes grown in the field. The fruits used were of known age. Once a week during the summer flowers that had just opened were tagged. This supplied me with fruits differing in age by one week, ranging from one week old to six, seven or eight weeks, or mature fruits.

In these first experiments fruits of different ages were selected, and no attention was paid to state of development or ripeness. Fig. 1 illustrates the results obtained with Livingston Globe. The John Baer fruits gave similar results, which were not quite as complete and for that reason are not included in this figure. In experiments A and B the youngest fruits were 8 and 9 days old, respectively, and the other fruits 1, 2, 3, 4, and 5 weeks older. It is to be noted that as the fruits become older the respiration decreases rapidly at first and then more slowly. Experiment C, which was conducted 4 days later than B, shows that the respiration of the youngest fruit was lower than in the preceding experiments but it agreed with what one would be led to expect from the curves A and B. Experiment D, which was conducted a week later than C, or when the youngest fruits were 3 weeks old had a rate consistently somewhat lower than expected, but this may have been due to a lower temperature.

[1] Paper from the Department of Botany of the University of Michigan, no. 301.

It will be noted that at no time was there an actual increase in respiration, but always a decrease. Several years ago HOVER and the writer noted that as leaves of sorghum, corn, oats and wheat matured, *i.e.*, aged, there came a period of minimum respiration after which there was an increase. I had expected to find a similar condition for fruits also but, as the curves show, it was not realized.

For a while it was thought that the difference in respiration rate of fruits of different ages might be due to size; *i.e.*, small fruits having a relatively larger surface than large fruits, might possibly respire more per gram of material than large fruits, which have a relatively smaller surface through which the absorption of oxygen and excretion of CO_2 takes place. In the fall and winter of 1927 experiments were therefore conducted to determine whether the size of the fruit exerted any influence on the rate of respiration, but nothing definitely was ascertained though in some experiments it seemed that large fruits respired less per gram of material than smaller fruits of the same age. Again, during the summer of 1928, this problem was taken up and this time the area of the surface was measured.[2]

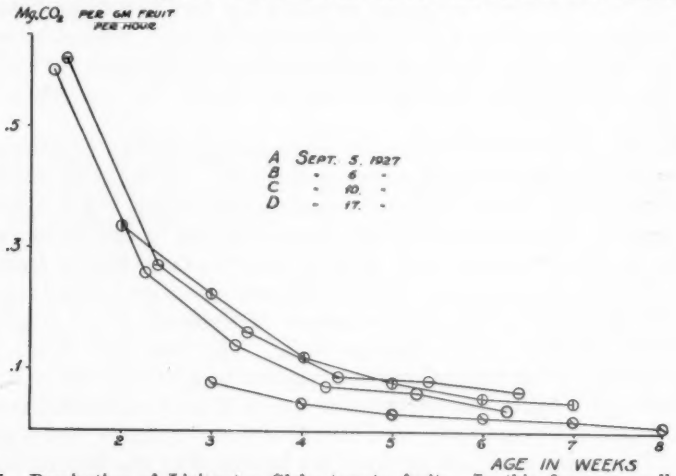

FIG. 1. Respiration of Livingston Globe tomato fruits. In this figure as well as in those to follow the green weight is used.

[2] The area of the surface was obtained by using SIMPSON'S 1/3 rule, where:

$$\text{Surface} = 4\ C \times 1/3\ S$$

S is one fourth of any circumference of the fruit passing through the point of stem insertion. C is the circumference of the fruit passing through points situated S distance from the center of the fruiting stem. The value thus obtained is 4.84 per cent. too large and has been corrected by that much reduction. I am indebted for this formula to Professor THEODORE R. RUNNING of the Mathematics Department of the Engineering School at the University of Michigan.

Fruits of the same age but having a different $\frac{\text{area}}{\text{weight}}$ ratio were used. When the weight of CO_2 produced was plotted against this ratio there was no uniformity and so far I have no evidence which would show that the increased surface exposed increases respiration. During the conduction of these experiments it was, however, discovered that although the fruits may be of the same chronological age, yet they may be of very different age physiologically. The recognition of this fact complicates the matter of relation between $\frac{\text{area}}{\text{weight}}$ ratio and rate of respiration, because it is next to impossible to get a number of fruits of exactly the same physiological age yet having different $\frac{\text{area}}{\text{weight}}$ ratios.

Finding that it would be very difficult to determine the effect of varying the $\frac{\text{area}}{\text{weight}}$ ratio on the rate of respiration this factor was eliminated with considerable success by choosing fruits of the same $\frac{\text{area}}{\text{weight}}$ ratio but of different physiological ages.

In the next experiments the chronological age was ignored, and fruits of approximately the same size but of different physiological ages were chosen. In this group of experiments only maturing fruits were used and after some study the physiological age of a fruit could be determined quite accurately from its color.

In fig. 2 are plotted the results of such experiments. The terms used on the abscissa are the color changes through which these fruits pass when ripening. It is realized that they are not very accurate but they are the best available. When 6 or 7 fruits differing only slightly in their physiological age, as shown by difference in color, are used in the same experiment, it becomes obvious that during the ripening period there is an increase in respiration which in turn is followed by a decrease as the fruits ripen still more. The increase is not very great in actual value but in terms of percentage it is quite considerable. Thus in experiment C the increase is 95 per cent., in D 75 per cent., while A and B show about 10 per cent. increase. These experiments were conducted in September, 1928. In the October number of the Proceedings of the Royal Society of London there appeared a paper by F. F. BLACKMAN (**2**) in which he describes experiments on stored apples, where the same phenomenon was noticed.

It is one thing to determine the physiological age or state of activity in a maturing fruit in which there are definite color changes and a very different thing to determine it in a green fruit. Age and size mean nothing. The way I have attempted to get at this is by measuring the diameters of the fruit at short intervals. In this way I have attempted to correlate rate of

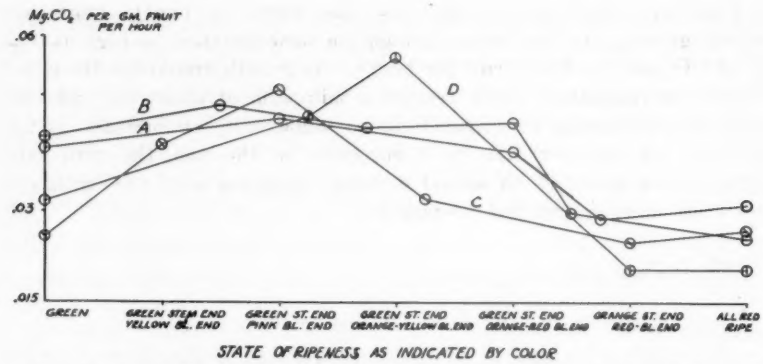

FIG. 2. Respiration of ripening John Baer tomato fruits, showing that as the fruits mature there is an increase in the rate of respiration, which is later followed by a decrease. The increase in respiration in experiments A and B was for each one about 10 per cent., for C 95 per cent. and for D 75 per cent.

respiration with the position a fruit occupied on the growth curve, but no two fruits have exactly the same growth curve and the placement of a fruit might be somewhat arbitrary, therefore in the calculations as shown in fig. 3 I have plotted amount of CO_2 against percentage increase in diameter per day of the fruits. Two diameters at right angles to the axis passing through the stem of the fruit were measured usually twice a week. From these data growth curves were plotted for each fruit and the fruits to be used in an experiment were selected with reference to their position on the growth curve.

In his paper on growth of the tomato MACDOUGAL (7) points out that the rate of increase in diameter is not a correct measure of actual growth, but rather that growth varies as the cube of the radius. Some of my data have been calculated in terms of percentage increase in the cube of the radius, but as the shape of the curve is the same, time has been saved by using the percentage increase in diameter in all experiments given in fig. 3.

When fruits for study were selected in this way curves like those represented by fig. 3 were obtained. These curves show a high rate of respiration when increase in diameter is large and a minimum when there is no longer an increase in size but the fruits are still green. This minimum was followed by an increase which reached its maximum at about the time when the fruits were of an orange to red color with the stem end still green. As in previous experiments with ripening fruit the actual increase is not very large but in terms of percentage it ranges from practically zero to 123 per cent.

These last experiments then show that when the tomato fruits are actively growing the respiration is high (in some instances as high as 0.75 mg. of CO_2 per gm. fresh fruit per hour). As growth diminishes there is a decrease in respiration which reaches a minimum at about the time the fruits stop increasing in diameter, to be followed by an increase, as the maturing process progresses, to a maximum at the time the fruits are orange to red in color. A second decrease continues until the fruits are ripe, when there is very little respiration.

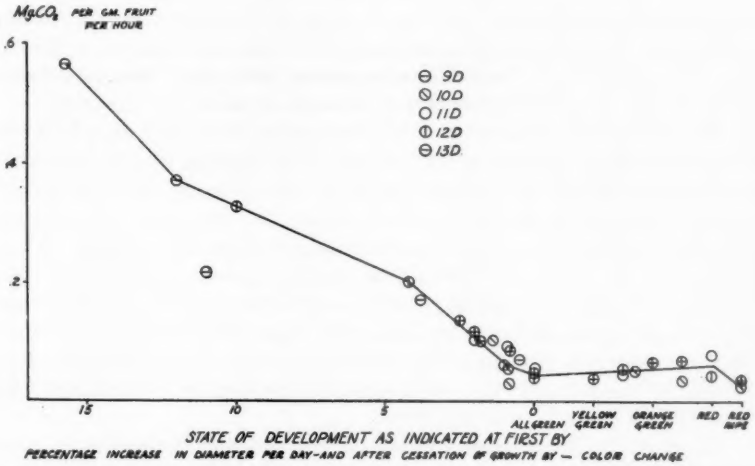

FIG. 3. Respiration of John Baer tomato fruits at different physiological ages.

These experiments showed that tomato fruits like leaves and apples have a period of minimum respiration which is followed by an increase as the fruits mature. Like apples, but as far as we know unlike leaves, this increase is further followed by a decrease.

It is to be expected that the young actively growing fruits will have a high respiratory rate, because respiration is so very closely connected with the life activity of an organism. It is also only to be expected that as the fruits become older, and growth slows down, respiration will diminish. It is, however, very hard to understand why there should be an increase in respiration after growth, *i.e.*, enlargement, has stopped. BLACKMAN explains this in the apple by assuming that as the fruits ripen there is a "lowering of the grade of organization-resistance" or more specifically a lowering of "hydrolysis resistance," which means that hydrolysis is facilitated during the ripening period. This increases the concentration of substances

utilized in respiration, and respiration increases. The subsequent decrease, he thinks, is due to starvation. Possibly there is starvation in apples that have been stored for several months, but certainly not in ripening tomatoes. Both ALBAHARY (**1**) and SANDO (**8**) have shown that there is an increase in sugars during the development of the tomato fruit.

According to Sando the percentage of total sugar in a ripe tomato is nearly twice that of a two-weeks-old fruit. He found that during the last two weeks there was an increase from 42.23 to 48.32 per cent. as calculated on the basis of dry weight or from 2.375 to 2.667 per cent. on the basis of green weight, and the red fruits have a sugar content which is two per cent. higher than that of the fruits turning color; but according to my findings the red ripe fruits with the higher sugar content respire less. In tomato therefore the final decrease in respiration cannot be due to starvation.

The decrease in respiration in the mature fruit might rather be attributed to complete cessation of all activity. The mature fruit is essentially water and carbohydrate. According to my own data (**3**) Livingston Globe 9 weeks old (nearly mature) contained only 4.6 per cent. dry material, John Baer 8 weeks old (mature) contained 5 per cent. dry material. SANDO found 8 weeks old (red) Livingston Globe to contain 5.5 per cent. dry material. Of this dry material carbohydrate made up 62.45 per cent., crude ash 9.14 per cent. and protein 13.13 per cent. Only 0.725 per cent. of the fresh fruit was protein and a great deal of this is in the seeds. In a system like this it is not very likely that life processes are very active. The whole system is slowly running down.

To return to the increase in respiration following the minimum point reached at about the time the green fruits stop enlarging, if augmentation in hydrolysability and the consequent augmentation in material utilizable in respiration is a factor in this increase of respiration it would seem that respiration should increase continuously from the beginning of the fruit development till the end, because, according to SANDO, the percentage of sugar content is constantly becoming greater, at least after the fruits are two weeks old. The percentage of protein is constantly diminishing, so respiration cannot be associated with that either.

In a recent paper (**4**) it has been shown that there is an increase in the hydrogen ion concentration as the fruits enlarge until a point of maximum concentration is reached, after which there is a decrease. In the experiments referred to, 50 fruits were used for each determination and these were average fruits of a given age, so that they varied in physiological age. At the time the maximum hydrogen ion concentration was reached in the fruits a few were beginning to turn but most were still green. This fact corresponds very well with the condition of the fruit at the point of minimum respiration. As it is a well established fact that respiration is

very greatly influenced by the hydrogen ion concentration (5) it seems to me that the explanation for the increase in respiration of the maturing tomatoes might be the decrease in the hydrogen ion concentration of the cell sap. This is merely a suggestion, but when the hydrogen ion concentration curve is compared with the respiration curve, it seems that this must be the correct answer. The origin of the acid substances need not concern us. They may be the result of the respiration itself, in which case we would have a self-regulatory reaction.

A word may also be said about the use of the physiological age, as contrasted with chronological age. As has been pointed out elsewhere, and as every one who has studied tomatoes knows, some fruits do not begin to develop at once after the flowers wither, but only after a week or two of inactivity. Physiologically these fruits are younger than the ones that started to grow at once. Frequently there are fruits in a cluster which, for one reason or another, get a slow start and grow very slowly until the other fruits are either mature or have been picked. Then they begin to grow very rapidly and consequently are much younger physiologically than chronologically. In a population of tomato fruits as they exist in a field one has to deal with such cases and for that reason age as measured in days and weeks is not a fair criterion of the behavior of a fruit. As far as I know this distinction has not been made in plant physiology, but it is one with which we are all familiar, at least in the human family. Not all people act their age.

Summary

1. It has been shown that there is a decrease in the production of CO_2 by John Baer tomato fruits during growth, until a point of minimum production is reached at about the time increase in size stops; that this is followed by an increase in CO_2 production which reaches its maximum when the fruits are orange to red in color and that thereafter there is a final decrease in the rate of respiration.

2. It has been suggested that the increase is due to a lowering of the hydrogen ion concentration of the cell sap.

3. The final decrease in CO_2 production is thought to be due to a slowing down of all life activities as the tomato approaches a condition of a mixture of carbohydrate and water.

4. It has been pointed out that sometimes there is a difference between the chronological and physiological age in tomatoes, and that the latter should be used when various activities are studied.

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SOME OBSERVATIONS ON THE MICROCHEMICAL DEMONSTRATION OF PHLORIDZIN[1]

E. M. HARVEY

(WITH THREE FIGURES)

MOLISCH (**2**), in his "Mikrochemie der Pflanze" (p. 162) states: ". . . Although the number of known glucosides in plants is exceedingly large, yet one is able to demonstrate but a few microchemically. In such tests one seeks either to demonstrate the specific glucoside directly, or, after hydrolysis, to demonstrate the non-sugar constituent."

The writer believes that, in the case of phloridzin, a direct demonstration is the more feasible, seeing that phloridzin and phloretin, its non-sugar derivative, have precisely the same color reactions. Arbutin, on the other hand, may give different color reactions than its derivative hydroquinone.

Several reagents for demonstrating phloridzin have been suggested. Among the best are concentrated sulphuric acid, nitric acid, and ferric chloride. The last named is an excellent reagent and, with a little experience, offers still the best aid to the investigator. The writer attempted to find some other reagent which would be more specific for phloridzin, but none distinctly superior to ferric chloride was found among the several hundred substances or combinations tried. Some, however, seemed worthy of consideration, such as uranium acetate (or chloride), mercuric nitrate, aluminium nitrate, antimony pentachloride, and sodium tungstate. These were tested not only upon phloridzin, but also upon nineteen other glucosides or glucosidal derivatives and tannins. The color reactions resulting were frequently rather distinct and characteristic. For example, with *ferric chloride,* phloridzin gives a clear orange to vinaceous red; tannic acid gives a deep blue or blue-black; arbutin, a blue also; salicin is colorless; and quercitrin produces a deep green. With *uranium acetate,* phloridzin is orange, while tannic acid and arbutin give with this reagent a vinaceous red. With *aluminium nitrate,* phloridzin gives a lavender-pink to vinaceous red; tannic acid, a clear green; and arbutin is colorless. The employment of two or more reagents, with strict attention to the color reactions should aid greatly in the identification of certain glucosides. One should also take into consideration the dominance of one reaction over another. To illustrate, it was found by testing certain solutions with ferric chloride that tannic acid dominated phloridzin, and the latter the arbutin reaction. If one part phloridzin is present to ten parts arbutin, the blue coloration of arbutin will not be evident. One part phloridzin to twenty or thirty parts

[1] Published with the approval of the Director of the Experiment Station.

arbutin will still interfere somewhat with the latter reaction. On the other hand, one part of tannic acid to three parts phloridzin entirely obscures the phloridzin reaction; but with one part tannin to six or seven parts phloridzin, a green coloration results.

The above statements indicate some of the factors which must be considered in making microchemical tests for glucosides.

The writer has made many more or less sporadic microchemical tests for phloridzin in various species of Rosaceae. Except for the apple, phloridzin could not be positively identified in any of the species examined. Nevertheless its presence was strongly indicated in our choke cherry, *Prunus demissa*. Phloridzin has been reported from the sweet cherry and prune and other Rosaceae besides the apple, but such observations seem at present uncertain. HERMANN (1) states: "In the pear, prune and cherry

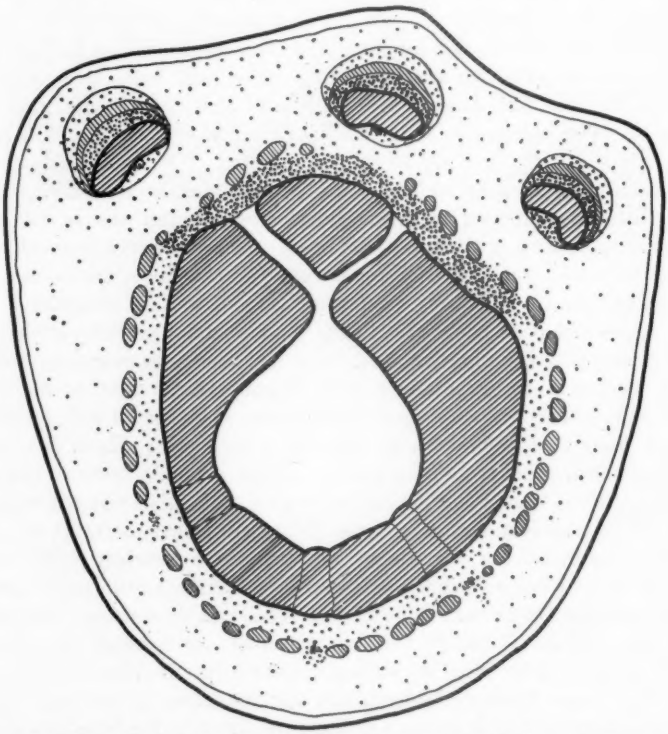

FIG. 1. Cross-section of apple shoot on March 16. Cut just below a leaf scar. The dots show the presence and relative amounts of phloridzin. Cross hatching indicates xylem and bast.

the phloridzin reaction does not occur, on account of the interference by tannic acid." But TUNMANN (see 1) comments on this interpretation: "Perhaps there is no phloridzin present."

In the apple the demonstration of phloridzin is a simple matter. Either ferric chloride or uranium acetate will serve as a reagent. A stock solution of ferric chloride (50 per cent.) will keep indefinitely. This is diluted to five, two, or even one per cent. for immediate use. These solutions may be used for direct examination of specific tissues by flooding cross or longitudinal sections on a slide, using a binocular microscope with strong illumination from above. Or, since the reaction is a "diffused" one, that

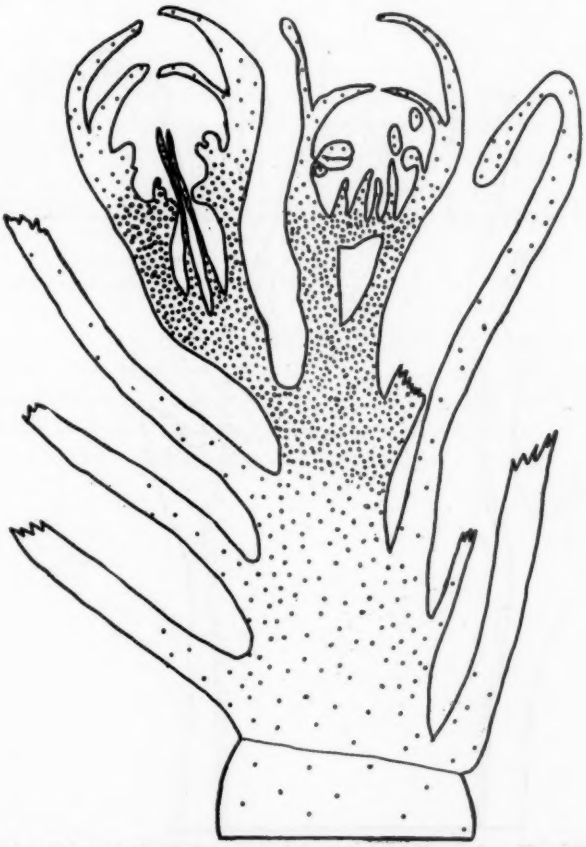

FIG. 2. Section through a bursting terminal fruit bud of the apple. The dots show the presence and relative amounts of phloridzin.

is to say one in which a vinaceous red cloud soon appears out from the margins of the sections, the fact may be taken advantage of in comparing different tissues. Representative pieces of outer or inner cortex or phloem, for instance, can be dissected out and tested separately, observing, on crushing in the reagent, the relative density and breadth of the diffusion clouds of phloridzin. As to the localization of phloridzin in apple tissue, HERMANN (**1**) reports the following: "Phloridzin is localized in the outer cortex, a little in the phloem region, and none in the cambium, bast or wood." Fig. 1 shows that this statement agrees fairly well with the findings of the writer. However, the following modification is suggested. Phlo-

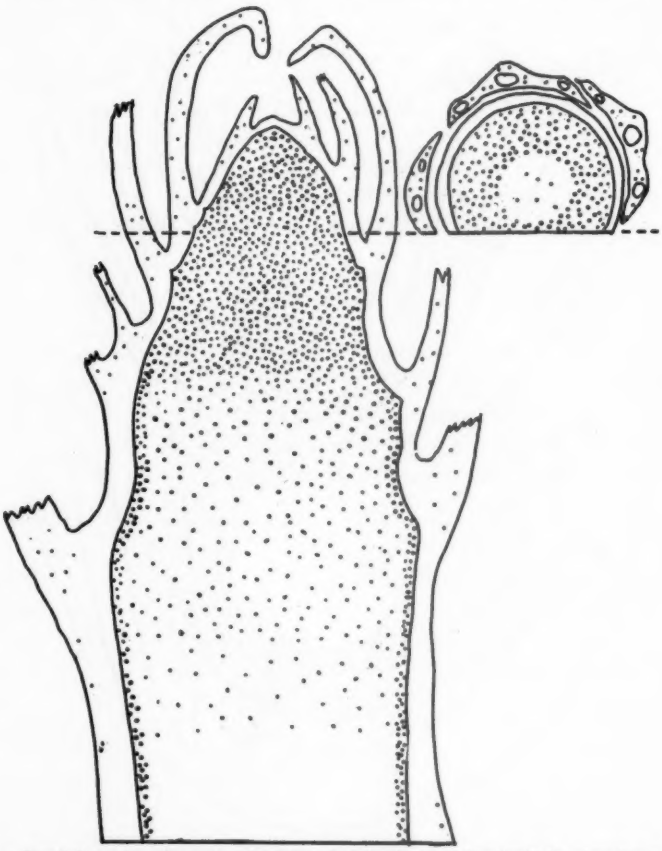

FIG. 3. Partial section through a bursting terminal vegetative bud showing the growing point. The dots indicate the presence and relative amounts of phloridzin.

ridzin is most abundant in the phloem, particularly the phloem of leaf traces, abundant in the outer cortex, or inner periderm, but rather scarce in the inner cortex, except at the nodes around the leaf traces, and if it does not occur in the cambium, it occurs very near it. Tannins cause the color reaction to be greenish in the outer cortex. In the bark of spurs and older branches, there is usually sufficient accumulation of tannins in the outer phloem rings to make the reaction unsatisfactory. Older roots, while containing large quantities of phloridzin, seldom give clear reactions on account of tannin.

At the beginning of growth activity in the spring, when buds are swelling or bursting, the bases of axillary and terminal buds are gorged with phloridzin. Then the inner bud scales, the peduncles, and receptacles of the embryonic flowers are also gorged with it (see fig. 2). A quantitative determination of phloridzin in stylar tissue showed, on a dry weight basis, 15.20 per cent. At the same time the new growth of the spur contained but 8.42 per cent. phloridzin and the spur bark of the previous year, 5.98 per cent. Large quantities were observed microchemically in the growing points of bursting buds (see fig. 3).

The conclusion seems justified that phloridzin is localized and abundant in, or in close proximity to, the most active tissue.

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THE EFFECT OF pH VALUE AND HYDROGEN PEROXIDE CONCENTRATION ON FRUIT OXIDASE ACTIVITY

W. V. Cruess and W. Y. Fong

In connection with our studies on the darkening of fruit tissue, investigations have been made on the effect of hydrogen ion concentration and hydrogen peroxide concentration on the behavior of the oxidase system of the apricot. Some of the observations that appear to be of general interest are presented in this paper.

Effect of pH value on the action of the oxidase on various indicators

Several investigators have proved that hydrogen ion concentration markedly affects the activity of various oxidases.[1] Among these may be mentioned Ucko and Bansi (**7**) who found that the pH optimum for the peroxidase of horse radish varied with the nature of the oxidase indicator, being pH 7 for pyrogallol and 5.2 for guaiacol; Clark and Zoller (**2**) who selected a number of oxidation and reduction indicators for oxidizing enzymes; Raper (**6**) who found that the maximum effect of tyrosinase or tyrosin occurs at pH 6–8 and that there is no action below pH 5 or above 10; and Overholser and Cruess (**5**) who found that the activity of apple oxidase on benzidine was affected markedly by the pH value of the medium. Overholser (**4**) finds that pear catalase is most active in the neighborhood of pH 7.0.

Our measurements on the effect of the pH value of the medium on apricot oxidase were made both upon the natural apricot juice and on the enzyme purified by precipitation with alcohol and reprecipitation by acetone from water solution. Incidentally, acetone was found to be an excellent precipitant for fruit oxidase as the precipitate was granular and easily washed and filtered.

Portions of apricot juice were brought to various pH values ranging from pH 2.0 to 11.1 and a number of different oxidase indicators were added, together with a small measured amount of 0.3 per cent. H_2O_2 solution. The peroxidase was found in most cases to be capable of oxidizing α-naphthol at pH 2.8 but not at 2.6; ortho-amino-phenol, para-amino-phenol, hydroquinon, pyrogallol, para-phenylene-diamin hydrochloride and benzidine at pH 2.6 but not at 2.4; para cresol at pH 2.9 but not at 2.6; guaiacol at pH 3.0 but not at pH 2.8; tincture of guaiac at 3.3 but not at 3.2. Tyrosin was not oxidized at any pH value under the conditions of the tests, *i.e.* on addition of a dilute solution of the indicator to the buffered sample

[1] We have followed the usual practice of considering a plant oxidase to consist of an organic peroxide and a peroxidase.

of juice. The optimum pH value varied with the different indicators. Allowing for atmospheric oxidation at high pH values, the optimum pH value for the oxidation of pyrogallol appeared to be at about 7; hydroquinon about 8–8.5; para-cresol and the ortho-and para-amino-phenols about 5.5–6; para-phenylene-diamin-hydrochloride at about pH 5 and guaiacol about 5.0 to 5.5. The necessary color comparisons were made with a Klett colorimeter. Because of the rapid fading of the oxidized color of tincture of guaiac it was difficult to establish its optimum pH, although it apparently colored by oxidation most rapidly at pH 5.0 to 8.0.

On the alkaline side of neutrality the darkening of the various indicators was inhibited at different pH values. Guaiacol failed to oxidize at pH 9.5, benzidine at 9.8, α-naphthol at about 10.0 and tincture of guaiac at 9.8; para-cresol and para-phenylene-diamin-hydrochloride between 9.2 and 11.5. The other indicators showed marked atmospheric oxidation at pH values above 7.8 and it was therefore difficult to establish their upper pH limits although above pH 9.2 the intensity of combined atmospheric and oxidasic oxidation rapidly decreased with increase of pH value.

It was apparent that the choice of indicator affected the pH maximum, minimum and optimum for apricot oxidase. Similar results were obtained with the peroxidase of peaches and pears. In general, oxidase action on all of the indicators was much less at low pH values than at those near neutrality, and decreased with decrease in pH value to a vanishing point between pH 2 and pH 3 for most of the indicators tested.

Effect of H_2O_2 concentration

BERNHEIM and DIXON (1) and WILLSTÄTTER (8) as well as others have reported that peroxidase activity is affected by the concentration of added H_2O_2. We found that the peroxidase of the apricot is similarly affected although the optimum and the maximum values varied somewhat with the indicator. By addition of the H_2O_2 and indicator to juice from cold stored apricots and peaches (very low in catalase activity) the optimum range for hydroquinon was found to be from 15–25 mg. per 100 cc.; for α-naphthol 5–10 mg.; pyrogallol, 5–10 mg.; and for guaiacol, ortho-amino-phenol and para-cresol approximately 10 mg. per 100 cc. At 480 mg. of H_2O_2 per 100 cc., the peroxidase reactions with α-naphthol and hydroquinon were negative and at 360 mg. were negative for pyrogallol. The optimum is naturally affected by the relative activity of the catalase which tends to rapidly destroy the H_2O_2 in juices rich in catalase. The catalase activity is affected by the pH value of the medium [see OVERHOLSER (4)]. The pH value of the apricot juice was approximately 4.0; that of the peach juice slightly less than 4.

Inactivation pH values for peroxidase

Apricot juice was brought by addition of tartaric acid, or sodium hydroxide and sodium bicarbonate to pH values ranging from 1.75 to 13.0. At intervals small portions of each sample were brought to approximately pH 7.0 and were tested with several peroxidase indicators. On the acid side of neutrality the peroxidase was apparently destroyed in 3 hours or less between pH 2.0 and 2.1, and on the alkaline side at about pH 12.8. At 18 hours the peroxidase was destroyed at about pH 12.1 and at 48 hours at about 11.7. The organic peroxide was at least temporarily utilized or inactivated at pH 8.5.

As previously reported (**3**) the temperature also affects the pH value necessary for inactivation; or rather conversely, the temperature necessary for inactivation varies with the pH value; the oxidase being most resistant to high temperatures near neutrality. Our earlier results were confirmed by quantitative colorimetric measurements using several oxidase indicators; the effect of heat at various pH values was found to be progressive rather than abrupt. These determinations were made both with the natural juice and with the peroxidase prepared by precipitation with ethyl alcohol and reprecipitation with acetone.

Summary

1. The optimum, minimum and maximum pH values for various oxidase indicators in contact with apricot oxidase vary with the indicator; but in general the optimum for most of the indicators used in these studies lies near neutrality.

2. The hydrogen peroxide concentration is not a matter of indifference, but greatly affects the intensity of the oxidase reaction with various indicators, the optimum range of H_2O_2 concentration in apricot juice low in catalase being from 5 to 25 mg. of H_2O_2 per 100 cc.

3. The peroxidase was inactivated at about pH 2.0 and at about 12.8 in less than 3 hours and on subsequent adjustment to neutrality failed to respond positively with the usual peroxidase indicators. The organic peroxide was considerably less resistant than the peroxidase to high pH values.

4. In general, in any comparative studies on the activity of fruit oxidase the pH value of the medium and the H_2O_2 concentration must be carefully controlled and the proper indicator chosen.

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TESTING THE SUGAR CONTENT OF BEETS FOR GENETICAL PURPOSES

ERNEST REED

Introduction

Since sugar beets are raised solely for sugar the one characteristic of prime importance is the sugar content. It has been shown (**6**) that some of the difficulties involved in the breeding of beets are matters of flower development and fertilization. PRITCHARD (**5**) claimed that there is no correlation between morphological characters and sugar content and further that sugar content of the beet is not inherited. Such studies are dependent upon exact control of pollination and a correct determination of sugar content in the beet. The purpose of this paper is to point out a source of error in the methods[1] now used in sugar beet testing and to present a method in which this particular error will not be possible.

There are various methods in use for sampling individual beets for sugar determinations (**1, 2, 3, 4, 7, 8, 9**). The simpler of these methods is to grind the beet pulp and extract the juice. Another method involves boring a hole through the beet and catching the pulp and juice as they ooze out. The underlying principle of these methods is to break the cells of the beet tissue. Lead sub-acetate is then added to the normal weight of the pulp and the sugar diffuses throughout the liquid because of the cells having been broken. Ten or more minutes are allowed for cold water digestion.

Theoretically, polarization of the filtrate from these preparations should give a relatively accurate reading of the percentage of sugar content in the beet. Actually, however, mechanical devices which it is necessary to use either in grinding or boring the beet cannot be relied upon to break all of the cells. Since hundreds, and often thousands, of beets are tested in this way the test for the group may vary in proportion to the efficiency of the grinding machine or the boring rasp which may be used in securing the pulp. The rasp may be in perfect cutting condition for the first beet but it naturally becomes duller as subsequent borings are made so that a variation in sugar content which is due to the method will make the data valueless.

Experimental

In connection with the work reported in this paper, six samples of pulp were secured from the same beet with a newly sharpened rasp made specifically for this purpose. Cold water digestion for thirty minutes was

[1] This work is concerned only with simple and rapid methods which may be used in the testing of several hundred beets a day by one or two persons.

allowed before filtration and the six samples were treated alike and polarized at the same time with the same polariscope. The six samples tested as follows:

1st	6.2	per cent.
2nd	6.7	" "
3rd	6.2	" "
4th	6.7	" "
5th	7.5	" "
6th	8.7	" "

We have here a range of from 6.2 per cent. to 8.7 per cent. in six tests from one beet, a variation of 2.5 per cent. sugar content or a possible error of 24.8 per cent. in the calculation. Some of the remaining pulp from these samples was examined with the microscope and it was found that quite a few of the cells were not broken. Similar tests were made from many other beets and a wide range of sugar content was secured for every beet thus sampled several times.

The essential thing in the cold water or warm water digestion methods is that every cell should be so broken down that the sugar from within may freely diffuse and so be represented in the filtrate which is polarized. The mechanical devices which are used in testing large numbers of beets which are necessary in beet breeding studies must of necessity introduce a large error, so large in fact that the data become useless for genetical analysis.

Twelve more samples were secured from the same beet mentioned above. Six of these samples were secured by the boring rasp and six others were secured as chunks cut out with a knife. The necessary lead sub-acetate water was added to each sample and all were placed in an autoclave. Here they were exposed to 15 pounds pressure for 15 minutes and then allowed to cool to room temperature. The filtrates from these samples were polarized and each gave the same reading, a 10 per cent. sugar content value.

In this study is involved ground pulp in six of the samples, and chunks of beet tissue in the other six. There are probably large numbers of unbroken cells in the pulp and the cells of the chunks are not broken except along the outside surfaces. The autoclave treatment breaks down the otherwise sugar impermeableness of the cells and so makes possible free diffusion of the sugar from all of the cells. If this is true the tests of each sample should be the same as that of any other sample and we see that this is the case.

But we also find that each sample that has gone through the autoclave gives a sugar percentage reading which is higher than the highest test from those samples which were not put through the autoclave. There is a difference of 1.3 per cent. between the autoclave sugar reading and the highest

non-autoclave sugar reading and the greatest difference between the autoclave and the non-autoclave samples is 3.8 per cent.

Subsequent studies, on many other beets, were made similar to the above, with the same fundamental differences occurring between the two methods, and also with all of the samples from any one beet testing the same when put through the autoclave, whether as pulp or as chunks.

The question which naturally arises is this. Does not the autoclave method so affect the carbohydrate contents of the beet sample as to make available more material which might be recorded on the polariscope along with and in addition to the sucrose?

A known amount of sugar (cube sugar) was added to certain samples and some of these were subjected only to the cold water digestion and others to the autoclave. There resulted no difference, which indicated that the autoclave did not affect the ordinary sugar which was added to the lead sub-acetate. This, however, did not provide against possible changes which might occur on carbohydrates in the beet tissue.

Finally the following method was used and seems to serve as a convincing check on the autoclave method.

Thirty samples were taken from the one beet and the correct amount of lead water added to each. Fifteen of these samples were put through the autoclave and the resulting sugar test was 15.8 per cent. in each case. The other fifteen samples were frozen at $-80°$ C. by the use of solid carbon dioxide. Here again, the test in each case was 15.8 per cent.

It is to be expected that the freezing method would slow down chemical reaction in the beet tissue to a minimum. The only changes occurring in the beet tissues thus frozen would be physical changes, such as might destroy the sugar impermeableness of the cells. The freezing method was repeated with twenty other beets and in each case an equal number of tests was made from each beet with the non-autoclave method and the autoclave method. The results were always the same, namely, the non-autoclave method gave a lower test than the other two methods. The autoclave and freezing tests checked and further the tests secured by the non-autoclave method for any one beet did not agree with each other, whereas the autoclave and the freezing method tests for any one beet were always the same.

Discussion

It has been pointed out by PRITCHARD (5) and others, that the data which have been secured from extensive studies in sugar beet breeding show no apparent correlation between any of the characteristics of the beet and the sugar content. There is a large amount of data at hand which indicates that sugar content is not a heritable characteristic. These interpretations of the data are not only justified but seem to be the only conclusions that it is possible to draw from the data.

There are two possible sources of error; that involved in fertilization, Reed (**6**), and that error which is due to the methods of pulp extraction for sugar testing, as explained in this paper.

When beets are tested for sugar content it is assumed, and rightly so, that when the cells of the beet are broken in water the sucrose will diffuse throughout the water. This water may then be polarized and the sugar percentage thus determined. Lead sub-acetate is used with the water as a clarifying agent.

It becomes apparent that the accuracy of the sugar test depends primarily upon the efficiency of the instrument which is to break down the cells. Any cells which are not broken will not only retain their sugar but will also take up water. There have been devised various rasps for boring through the beet. These rasps are so provided with cutting teeth that they are expected to cut every cell of the beet pulp. This pulp is not further treated in order to insure against any of the cells not being broken but it is weighed out and subjected to cold water digestion. Any unbroken cells are not detected and the extent to which unbroken cells may occur in this pulp will be registered in the percentage reading as an error.

This possibility of error was demonstrated to be very great by the microscopical examination of pulp which was secured by the use of the rasp. The occurrence of this error was definitely demonstrated by the differences between the tests which were made on several samples from the same beet.

With this source of error in mind, the accumulated data, which have been used to draw the conclusions that sugar content is not inherited, and that there is no correlation between phenotypic characteristics and sugar content, become useless and the conclusions are of no value. Knowing the extent of error which is likely to be involved in such work, it is to be expected that sugar percentages secured by the use of these methods shall vary greatly and be of no use for genetical analyses.

By use of the autoclave, as described in this paper, one may secure accurate sugar percentage readings of individual beets. The method is simple, the operations are more easily and more quickly performed, as it is neither necessary to grind the beet tissue nor to bore it. The theory underlying this method is the same as that of the other methods but in this case the sugar permeableness of the cells is guaranteed by the effectiveness of the autoclave treatment.

The accuracy of the autoclave method is demonstrated by the fact that the several samples taken from the same beet test the same when this method is used, and by the fact that the tests which are secured by use of the autoclave method check with those of the freezing method. The data presented earlier in this paper show that the total sugar is not recorded

in any sample secured by use of the rasp, whereas it is apparent that the autoclave method insures a test of all of the sugar of the sample. This statement is substantiated by the fact that the tests by the use of the autoclave method agree with those of the freezing method.

By using solid carbon dioxide to freeze the tissue, we have a reliable check on the autoclave method. Some such check is necessary because our knowledge of the changes which may take place in beet tissue in the autoclave is not sufficient.

Summary

The following method is recommended for determining the sugar content of individual beets where such is desired for genetical study, where accuracy is essential.

The normal weight (26.048 grams) of the beet pulp is put into a lead subacetate solution (thirty baume) 177 cc. This is autoclaved at 15 pounds for 15 minutes and allowed to cool to room temperature. The solution is then filtered and the filtrate polarized.

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EFFECT OF ACID AND ALKALINE HYDROLYSIS ON THE ESTIMATION OF HEMICELLULOSE AND ASSOCIATED GROUPS IN YOUNG APPLE WOOD

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The greater portion of the food reserve and structural network in plants is composed of carbohydrate material. In the vegetative tissue and seed of many plants, there exists a group of hexosan-pentosan polysaccharides to which SCHULZE has given the name of "hemicelluloses." These hemicelluloses are easily hydrolyzed by dilute acids into simple hexose and pentose sugars or may be isolated from the tissue in the colloidal form by means of weak caustic solutions. The characterization of this group of substances is even less specific than our present definition of cellulose. This apparent indefiniteness of classification is due partially to the fact that neither of the above methods of isolation effects a sharp separation of cellulose from lignin and the associated hemicellulose groupings.

As a means of orientation in the general problem of investigating the distribution and function of these polyhexose-pentose structures in the metabolism of the plant, it became of interest to study the two usual methods of their determination, and further to note the effect of such procedures upon the tissue components associated with them. An attempt was also made to identify the sugars present in the hemicellulose fraction.

No effort will be made here to review the voluminous literature pertaining to the distribution of these anhydride sugars in plants. The earlier work of SCHULZE (**27, 28**) has been enlarged through the efforts of O'DWYER (**22, 23, 24**), CANDLIN and SCHRYVER (**3**), NORRIS and SCHRYVER (**21**), and LING and NANJI (**15**) in a series of papers dealing with the isolation, properties and constitution of the hemicelluloses. KARRER (**13**) found a reserve cellulose (lichenin) present in lichens which yielded only glucose upon hydrolysis. O'DWYER (**23**) and EHRLICH (**7**) have shown that hemicelluloses are not true carbohydrates (pentosans, hexosans), but also contain acid groups of the glucuronic type and are more nearly like the pectins than like true cellulose. VON FELLENBERG (**33**) EHRLICH (**7**) and CANDLIN (**3**) suggest the possibility that hemicelluloses may prove to be the intermediate stage between pectin and lignin because of the fact that unlignified tissues have a larger proportion of pectins than hemicellulose, while in the lignified material the opposite is true. The transition of pectin into hemicellulose is presumed to occur through a process of decarboxylation (**24**).

In the resolution of the hemicelluloses during acid or alkaline hydrolysis certain changes are produced in the residual tissues. These changes may alter the composition of the hemicellulose fraction. NORRIS and SCHRYVER

(**21**) have shown that the method of pectin removal influences the composition of hemicellulose, while TOTTINGHAM and GERHARDT (**32**) and O'DWYER (**23**) found that the kind, strength, and duration of hydrolysis also exerts a specific effect. According to SHERRARD (**30**) weak acid hydrolysis destroys over 30 per cent. of the cellulose, 83 per cent. of the pentosans, and over 16 per cent. of the methoxy-groups in wood. PETERSON and HIXON (**25**) have shown that the alkaline extraction of the hemicellulose in the cornstalk also removes large amounts of lignin. HAWLEY and CAMPBELL (**11**) state that partial hydrolysis effects definite changes in wood similar to those produced by certain white and brown-rot fungi (**10**). These changes involve a destruction of the pentosan and cellulose fractions, and increase the alkali solubility (lignin) of the wood.

Material and methods

One year old apple wood was used as the experimental material. This was collected as one large sample (one kilo dry matter) on April 10, 1928. After removal to the laboratory the tissues were cut into small pieces and desiccated under reduced pressure at 70° C. The material was then ground to pass a 60 mesh sieve. The air-dried wood contained 4.75 per cent. moisture and 3.47 per cent. ether soluble material.

Lignin and cellulose were determined by treatment of the tissue with 4 per cent. NaOH at 180 pounds pressure for one hour according to the method of MEHTA (**16**).

Methoxy-groups were identified by the ZEISEL method as modified by DORE (**6**).

Pentosan determinations were made by distillation with 12 per cent. HCl according to the Official Methods (**1**).

"Uronic" acids were determined by precipitation of the liberated carbon dioxide with $Ba(OH)_2$ as specified by NANJI, PATTEN and LING (**19**).

Pectins were removed by extraction of the tissue with 0.50 per cent. $(NH_4)_2C_2O_4$ according to CLAYSON, NORRIS and SCHRYVER (**4**).

Dilute acid hydrolysis was accomplished by boiling the residual tissue after pectin removal with 2.50 per cent. H_2SO_4 (by weight) for 2 hours.

Dilute alkaline extraction of the hemicellulose fraction from the tissue after pectin removal was accomplished by treatment with 4 per cent. NaOH at room temperature for 24 hours (**23**).

The hot water extract represents that fraction of the tissue removed by boiling during the enzymatic digestion of the starch.

Experimental

COMPOSITION OF THE TISSUE AS AFFECTED BY PRELIMINARY TREATMENT

DORE (**5**) has shown that partial hydrolysis affects the more residual components of the plant cell, especially the methoxy-content of the lignin

and the furfural yielding constituents of the cellulose. In the following procedure, young apple wood was subjected to certain extractive and hydrolytic treatments. Analyses were made of the untreated and the residual tissues, and a comparison of these data gives an indication of the specific effect of each procedure.

The percentage of tissue removed during the different specific treatments was determined as follows: four aliquot samples were freed from hot water-soluble constituents including starch, and dried to a constant weight. Two samples were then used for analysis of the tissue, while the remaining two were further extracted with ammonium oxalate for removal of pectin, and after desiccation to constant weight, were again subjected to analysis. The acid and alkaline treatments for hemicellulose removal were confined to samples which had previously been freed from water-soluble, starch, and pectic materials. The percentage of tissue accounted for represents the summation of the hot-water extract, ammonium oxalate extract, loss on dilute acid hydrolysis, pentosan-free cellulose, pentosan in cellulose, pentosan destroyed during the cellulose determination, lignin and ether extract. These data are assembled in table I.

The data in table I indicate that preliminary treatment produces a marked change in the approximate analysis of the wood, hot water alone removing 6 per cent. of the pentosan and sugar acids of the glucuronic type. Removal of the pectic constituents with $(NH_4)_2C_2O_4$ produces little change in cellulose, lignin, or methoxy-content. However, about one half of the total "uronic" acids and 20 per cent. of the total pentosans in normal apple wood are associated with pectic compounds, the structural formula of pectin as suggested by NANJI (**19**) presupposing the presence of galactose, pentoses and sugar acids.

Decided differences in composition of the residual tissue are produced by hemicellulose removal by either acid or alkaline hydrolysis. Both methods remove approximately the same amount of total material but their specific effect upon the residual tissues is quite different. Acid treatment reduces the yield of cellulose by almost 30 per cent. and reduces the residual pentosan therein by over three-fourths. Its action upon lignin is largely confined to a hydrolysis of its methoxy-groups, since the loss in methoxy and total lignin approach a common value. Approximately one-half of the total pentosans in the tissue is removed, while the "uronic" acids are affected but little. The latter fact is an indication that about one-half of the CO_2 liberated and calculated as "uronic" acids has its origin in the pectic constituents, while the greater portion of the remainder is found, not in the hemicellulose fraction, but rather associated with the more residual skeletal components. The loss in cellulose due to acid treatment is largely

TABLE I

A. Percentage composition of 1 year old apple wood before and after various specific treatments (moisture-free basis)

Specific treatment	Cellulose	Lignin	Pentosans in cellulose calculated to original wood	Total pentosans in tissue	Methoxy	"Uronic" acids	Percentage of tissue removed	Percentage of tissue recovered
Normal wood, untreated	25.96	12.50	2.70	19.46	3.60	2.54	0.00	98.11
Extracted with hot water (starch free)	25.86	12.00	2.70	18.12	3.57	2.37	23.40	97.96
Extracted with ammonium oxalate	25.32	12.00	2.63	15.44	3.30	1.25	7.60	97.79
Hemicellulose removed by acid hydrolysis	18.76	10.88	0.65	7.33	3.07	1.14	21.00	91.79
Hemicellulose removed by alkaline hydrolysis	23.40	6.86	1.54	6.86	2.85	1.08	18.47	88.50

B. Percentage loss of tissue components after various specific treatments

Extraction with water	0.38	0.41	0.00	6.88	0.88	6.77		
Extraction with ammonium oxalate	2.46	0.41	1.51	20.65	7.81	47.26		
Hydrolysis with acid	27.70	9.70	75.90	52.66	8.17	9.60		
Hydrolysis with alkali	9.86	43.07	42.90	55.50	13.63	12.80		

accounted for by an increase in the beta and gamma forms (**16**) in the liquors from the cellulose determination. It is interesting to note that even such a drastic acid preliminary treatment does not remove completely the residual pentosan complex from the cellulose. The furfural value derived from the cellulose is due to pentosans and not to oxycelluloses because no characteristic color reactions or decarboxylation of the cellulose, indicating the presence of such groups, could be obtained.

Resolution of the hemicellulose by alkali produces no great destructive action on the cellulose residue. However, at room temperatures it removes almost one-half of the total lignin of the wood. In this case the methoxy content is not destroyed in proportion to the total lignin. The lignin must be removed either as a straight solubility product or by hydrolysis and removal as the soluble sodium salt of lignic acid. Weak alkali removes approximately 3 per cent. less tissue than does acid hydrolysis. The percentage recovery of total tissue is also lower in the alkaline extracted wood.

Carbohydrate recovery in the acid and alkaline hydrolyzates

During the usual carbohydrate analysis of plant tissue, the hemicellulose fraction is estimated by a direct weak acid hydrolysis. Since acid and alkali remove almost like amounts of total tissue, it became of interest to determine whether the reducing sugar values and furfural yields also bore a similar relationship.

Two 25-gm. samples of apple wood were freed from starch and water-soluble materials. Pectin was removed by 0.50 per cent. $(NH_4)_2C_2O_4$. One such residue was refluxed on an electric hot plate with 2.50 per cent. H_2SO_4 for a period of 2 hours. The other residue was extracted with 4 per cent. NaOH at room temperature in a shaking machine. The filtered alkaline extract was neutralized and brought to an acid concentration of 2.5 per cent. H_2SO_4 and refluxed for 2 hours. The clarified hydrolyzates from both pro-

TABLE II

Carbohydrate recovery in the hemicellulose of young apple wood

Treatment applied to tissue	Reducing power in equivalents of glucose	Pentosan	"Uronic" acids	Total tissue removed
	per cent.	*per cent.*	*per cent.*	*per cent.*
Acid hydrolysis	10.61	10.76	0.21	21.00
Alkaline extraction	7.20	8.40	0.19	18.60

cedures were brought to volume and their composition based upon the original moisture-free wood is shown in table II.

Although dilute acid removes 21 per cent. of the original tissue, only approximately one half of this amount can be recovered in the form of sugar as shown in table II. The similarity of reducing sugar and pentosan values indicates that the hemicellulose fraction of young apple wood is polypentose in nature. TOTTINGHAM (**31**) has reported the presence of polyhexosans in the base wood of the apple. His technique, however, did not include the removal of the pectic constituents prior to acid hydrolysis. The amount of "uronic acids" in the products of hydrolysis is exceedingly small, over one half of the total amount present in the wood having been removed with the pectic material in the ammonium oxalate extract.

The reducing power and pentosan value of the alkaline hemicellulose extract are considerably lower than in the acid hydrolyzate. Either the 4 per cent. NaOH destroys some of the carbohydrate material, or its action upon the tissue is less drastic. When the tissue residue from the 4 per cent. NaOH extraction was further treated with boiling 2.5 per cent. H_2SO_4 for 2 hours, the hydrolyzate contained an additional reducing power of 4.60 per cent. and a pentosan value of 4.10 per cent. The carbohydrates from this second hydrolysis are also derivatives of polypentose material and imply a similar origin in the tissue. These results indicate that alkaline extraction liberates less of the hemicellulose fraction from the tissue than does direct acid hydrolysis. WINTERSTEIN (**34**) however, has stated that 5 per cent. alkali probably modifies the residual cellulose so that it will again respond to subsequent dilute acid hydrolysis.

ISOLATION AND ANALYSIS OF THE HEMICELLULOSE FROM YOUNG APPLE WOOD

After removing the starch with a saliva digestion, 200 gm. of the tissue were extracted with 1 liter of 0.5 per cent. $(NH_4)_2C_2O_4$ at 85° C. for 24 hours to insure complete removal of pectic materials. Proteins, coloring matter, etc., were removed with 1 per cent. NH_4OH. The residue from the NH_4OH extract was further treated with 1 liter of 4 per cent. NaOH at room temperature for 24 hours. The filtered alkaline extract was neutralized with acetic acid and the hemicellulose precipitated by the addition of an equal volume of 95 per cent. alcohol. After washing thoroughly with 60 per cent. alcohol, the precipitate was again brought into solution in 1 per cent. NaOH, reprecipitated with acetic acid and alcohol. It was washed with gradient strengths of alcohol and ether and finally dried over night at 100° C. A yield of 17.2 gm. (8.60 per cent.) of a light gray amorphous material was obtained. It had the following composition:

	Per cent.
Moisture	5.78
Reducing sugar	69.00
Lignin	14.28
Ash	5.19
Total	94.25

The furfural yield of this substance in equivalents of pentosan, amounted to 68.5 per cent. The product was laevorotatory $[\alpha]^{25}_{D} = -104°$ in 1 per cent. NaOH. The reducing power and pentosan value again indicate its polypentose nature.

Hydrolysis and identification of the sugars in the hemicellulose fraction

Three 2-gm. samples of the purified hemicellulose were hydrolyzed with 2.5 per cent. H_2SO_4 for a period of 3 hours. In each case after hydrolysis there remained an insoluble residue which amounted to 5.60 per cent. of the original sample. The three hydrolyzates were digested with $Ba(OH)_2$, clarified with neutral $Pb(C_2H_3O_2)_2$ and concentrated under reduced pressure to a volume of 25 cc. and a concentration of 7.20 per cent. The three hydrolyzates, using a 200-mm. tube, gave an average reading of $+13°$ on the Ventzke sugar scale, or a specific rotation $[\alpha]^{25}_{D} = +31.3°$. Aliquot portions had a reducing power in equivalents of glucose of 69.00 per cent.

In view of the fact that the reducing sugar and pentosan values are of approximately the same magnitude, it appears probable that pentose sugars alone are present. This situation is further substantiated by the failure to identify the presence of galactose through oxidation to mucic acid (**14**) or of mannose by its insoluble phenylhydrazone (**8**), or of ketohexoses by the diphenylamine (**12**) Pinoff (**26**) or Seliwanoff (**29**) test for fructose, or of glucose through oxidation to saccharic acid (**9**). These results are strong evidence that hexosans are not present in this material in significant amounts.

Five grams of the purified amorphous grey hemicellulose substance were again hydrolyzed, de-sulphated and clarified as previously described. The filtrate was concentrated under partial *vacuo* and purified according to the method of Morrow (**17**). Under no circumstances, however, was it possible to effect crystallization of the sugars either from alcohol or glacial acetic acid. An aliquot portion of the syrup was treated with 1 gm. of phenylhydrazine hydrochloride and 1.5 gm. of sodium acetate in a test-tube in a boiling water bath according to Mulliken (**18**). An osazone in the form of an orange-yellow mass appeared after 8–10 minutes' heating. The surface of the solution also contained oily drops. Microscopical observa-

tion and melting-point determinations identified the recrystallized product as the osazone of xylose. This sugar was further identified by oxidation with bromine and the formation of the characteristic crystals of the double cadmium salt of xylose. As oily drops in the osazone reactions are an indication of arabinose, an attempt was made to obtain its diphenylhydrazone by treating another aliquot of the syrup with an excess of diphenylhydrazine according to NEUBERG (**20**). After cooling the solution, a white crystalline hydrazone appeared. It melted at 207° C. and must represent the hydrazone of arabinose.

Taking MORROW's (**17**) value for the specific rotatory power of d-xylose, namely $[\alpha]^{25}{}_{D} = +19°$ and that of l-arabinose as $[\alpha]^{25}{}_{D} = +104$, a mixture of 7 mols. of xylose with 1 mol. of arabinose would have a specific rotation $[\alpha]^{25}{}_{D} = +29.6°$ a number in fair agreement with the mean observed value of $[\alpha]^{25}{}_{D} = +31.3°$ for the sugar mixture in the hemicellulose fraction. Furthermore, 8 mols. of such a mixture having an empirical formula of $C_5H_{10}O_5$ should contain 7 mols. of d-xylose or 87.5 per cent., and 1 mol. of l-arabinose or 12.5 per cent. According to BROWN (**2**) the percentage of sugars in a mixture may be calculated by combining the polariscopic and reducing sugar values in a simultaneous equation. Calculated by this method, the sugars in the hemicellulose fraction were found to be composed of 88.4 per cent. d-xylose and 12.7 per cent. l-arabinose,—figures in close agreement with the theoretical value. An attempt was made to quantitatively determine the arabinose present in the syrup according to NEUBERG (**20**). 10 cc. of the syrup corresponding to 0.720 gm. of the original hemicellulose or 0.497 gm. of sugar therein, when treated with a slight excess of diphenylhydrazone, yielded 0.144 gm. of arabinose diphenylhydrazone or 0.068 gm. arabinose which is equal to 13.70 per cent. of the total sugar present. Although both the observed and calculated values do not agree exactly with theory, they serve to indicate the molal ratio in which the two sugars are probably present.

Summary

A study has been made of the action of several extractive and hydrolytic procedures upon young apple wood during the isolation of the hemicellulose fraction. Hot water and salivary digestion removed 23 per cent. of the tissue. Its action upon the residual tissue was slight. Ammonium oxalate removed 7 per cent. (largely pectic constituents) of the tissue. About one-fourth of the pentosans and one-half of the "uronic" acids were removed in this fraction. Dilute acid hydrolysis removed 21 per cent. of the ammonium oxalate extracted residue. It also removed 10 per cent. of the lignin, 50 per cent. of the remaining pentosans and altered 30 per cent. of the cellulose. 4 per cent. NaOH at room temperature removed less car-

bohydrate and total tissue than did the acid treatment. It, however, removed almost one-half of the total lignin.

The isolated hemicellulose fraction of a 1-year-old apple wood has been purified and analyzed. It contained approximately 15 per cent. lignin, 70 per cent. pentose sugars, 5 per cent. ash, and 5 per cent. moisture. No hexoses were identified therein, d-xylose and l-arabinose were present, probably in a molal ratio of 7 to 1 respectively. 88 per cent. of the total reducing sugar present in this fraction was d-xylose and the remaining 12 per cent. was found to be l-arabinose.

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INFLUENCE OF SHAPE ON THE CHEMICAL COMPOSITION OF POTATO TUBERS[1]

J. J. WILLAMAN[2] AND ALICE M. CHILD

Decided differences in the shape of potato tubers of different varieties are a matter of common knowledge. It has been suggested that these variations in shape may affect the composition of the tubers. The evidence on this, however, is conflicting.

GLYNNE and JACKSON (**3**), investigating the distribution of dry matter and of nitrogen in potato tubers, dissected out the different zones and analyzed them. The main regions are the medulla, or inner, and the cortex, or outer region. They found that the percentage of dry matter is lowest in the skin, increases in the cortex, and then decreases in the medulla. The nitrogen content in these three is in the reverse order to that of the dry matter. COUDON and BUSSARD (**1**) obtained practically the same results.

EAST (**2**) states that the flatter tubers have a higher starch content, because of a greater proportion of cortex. Thus, if changing the shape changes the ratios of the zones, and if the latter differ in composition, it is fair to predict that the composition of the tuber as a whole will vary with the shape. On the above lines of reasoning WILLAMAN and WEST (**8, 9**) concluded that the more spherical tubers would have the higher protein content.

RENSKI (**6**), however, found a relation opposite to that above. He reports that the longer tubers have the lower starch content. His material included many varieties over a period of several years. More recently NEUMAN (**5**) also reports that the longer tubers have the lower starch content. He states that high nitrogen fertilization causes an elongation of the tubers, but he does not give any data on the nitrogen content of these tubers.

During 1922 and 1923 several lots of potatoes, including several varieties, were measured as to shape, and analyzed for dry matter and nitrogen. Although the data are somewhat scanty, they point rather definitely to the conclusion that the more spherical tubers have the higher protein content. Since it is improbable that the writers will have further data in the near future, it was thought best to publish the information so far obtained.[3]

[1] Published, by permission of the Director, as paper no. 868, Journal Series, Minnesota Agricultural Experiment Station.

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[3] Acknowledgment is due to Sister M. BERNADETTE LIES, now at the College of St. Theresa, Winona, Minn., and to H. O. TRIEBOLD, now at State College, Pa., for the analytical data used in this paper.

Methods and materials

From 12 to 20 tubers were taken for a sample for analysis. The dimensions were taken in millimeters, and averaged for each lot of tubers. The tubers were scrubbed, dried, and weighed on a special balance in air and in water for determining specific gravity. Then they were shredded for analysis. Dry matter was determined on a small sample by drying at 100° C. Protein was determined on the dried and finely ground material by the Kjeldahl-Gunning method.

The five groups of samples involved were as follows, all of them grown in Minnesota:

A. Fourteen lots of Rural New Yorkers, grown in various parts of Minnesota in 1922.

B. Eight lots of Irish Cobblers grown in 1922 on peat, with various fertilizer treatments.

C. One lot each of King, Irish Cobbler, Green Mountain, Rural New Yorker, Burbank Russet, Bliss Triumph, Russet, and Early Ohio, grown on completely fertilized peat in 1922.

D. Eleven lots of Early Ohios, grown in various parts of Minnesota in 1922.

E. Twenty-six lots of Early Ohios, grown in various parts of the state in 1923.

Analysis of the results

Group E, of 26 samples, is the only one of any size for treatment statistically; since even this number is too small to justify the use of the ordinary coefficient of correlation, the coefficient of correlation by rank was calculated, using the following formula taken from JACKSON (4):

$$r = 1 - \frac{6 \sum D_k^2}{n(n^2 - 1)}$$

and the probable error by the formula:

$$e = 0.706 \frac{1 - r^2}{\sqrt{n}}$$

taken from WHIPPLE (7).

The results of these calculations for group E are given in table I. As has always been found by other workers, there is a high positive correlation between specific gravity and dry matter. Neither of these factors, however, appears to be related to nitrogen content or to any of the shape ratios in these samples. The nitrogen content of the fresh samples is negatively correlated with the length-depth ratio and with the width-depth ratio. In other words, the depth of the tuber is the deciding dimension; and as the depth increases, the percentage of nitrogen in the tuber as a whole increases.

TABLE I

COEFFICIENTS OF CORRELATION (BY RANK) AMONG SEVERAL FACTORS IN EARLY OHIO TUBERS (1923)

$n = 26$

	DRY MATTER	SPECIFIC GRAVITY	NITROGEN, FRESH BASIS	NITROGEN, DRY BASIS	LENGTH / WIDTH	LENGTH / DEPTH	WIDTH / DEPTH
AVERAGE WEIGHT	-0.439 ± 0.112	-0.158 ± 0.135	-0.537 ± 0.098	-0.393 ± 0.117	$+0.474 \pm 0.107$	$+0.640 \pm 0.082$	$+0.171 \pm 0.134$
DRY MATTER		$+0.742 \pm 0.062$	$+0.523 \pm 0.100$	-0.105 ± 0.137	-0.141 ± 0.136	-0.101 ± 0.137	-0.163 ± 0.134
SPECIFIC GRAVITY			$+0.363 \pm 0.120$	-0.158 ± 0.136	-0.019 ± 0.138	-0.011 ± 0.138	$+0.009 \pm 0.139$
NITROGEN FRESH BASIS					-0.182 ± 0.134	-0.333 ± 0.123	-0.345 ± 0.122
NITROGEN DRY BASIS					-0.111 ± 0.137	-0.167 ± 0.134	-0.282 ± 0.127
LENGTH / WIDTH						$+0.903 \pm 0.025$	-0.086 ± 0.137
LENGTH / DEPTH							$+0.421 \pm 0.114$

TABLE II

FOUR LOTS OF POTATO TUBERS SEGREGATED INTO "LONG" AND "SHORT" GROUPS ON BASIS OF LENGTH-WIDTH RATIO

CLASSIFICATION	RURAL NEW YORKER	IRISH COBBLER	EARLY OHIO	EARLY OHIO
	1922	1922	1922	1923
Group	A	B	D	E
No. of samples—				
Total	14	8	11	26
"Long" class	5	4	6	11
"Short" class	9	4	5	15
Length-width ratio—				
Total range	1.28–1.07	1.15–1.04	1.47–1.21	1.43–1.27
Mean of "long" class	1.27	1.12	1.44	1.40
Mean of "short" class	1.15	1.07	1.26	1.33
Difference, per cent.	−9	−4	−12	−5
Width-depth ratio—				
Total range	1.34–1.20	1.34–1.25	1.14–1.10	1.15–1.09
Mean of "long" class	1.27	1.30	1.12	1.12
Mean of "short" class	1.24	1.29	1.12	1.13
Difference, per cent.	+2	0	0	+1
Length-depth ratio—				
Total range	1.64–1.36	1.54–1.34	1.66–1.35	1.61–1.42
Mean of "long" class	1.60	1.47	1.63	1.57
Mean of "short" class	1.43	1.38	1.42	1.50
Difference, per cent.	−10	−6	−13	−4
Dry matter—				
Total range, per cent.	25.7–20.0	22.8–17.9	25.9–22.3	24.4–20.7
Mean of "long" class, per cent.	23.4	20.2	24.3	21.9
Mean of "short" class, per cent.	22.5	21.7	23.8	22.6
Difference, per cent.	−0	+7	−2	+3
Nitrogen, wet basis—				
Total range, per cent.	0.543–0.350	0.518–0.326	0.529–0.410	0.499–0.377
Mean of "long" class, per cent.	0.400	0.416	0.456	0.422
Mean of "short" class, per cent.	0.457	0.436	0.479	0.434
Difference, per cent.	+14	+5	+5	+3
Nitrogen, dry basis—				
Total range, per cent.	2.50–1.61	2.27–1.53	2.21–1.72	2.17–1.73
Mean of "long" class, per cent.	1.77	1.99	1.88	1.92
Mean of "short" class, per cent.	1.98	2.00	2.03	1.94
Difference, per cent.	+11	0	+8	+1

This relation is not so evident when the nitrogen in the dry matter is taken for comparison.

Among the shape ratios, the length-width bears a very marked relation to the length-depth; and the latter bears a significant relation to the width-depth ratio. Thus the length is the dominating dimension. Furthermore, in comparing the weight of the tubers with the shape, it is evident that as the tubers increase in size it is largely because of increased length. This is to be expected from the fact that the newly formed tuber is almost spherical. It is surprising, however, that these data indicate less dry matter in the larger tubers. This is inconsistent with the insignificant coefficient of correlation between size and specific gravity. The larger tubers apparently have a lower nitrogen content, which is consistent with the low nitrogen and long tubers. A corollary of the above facts is that the highest nitrogen content may be expected in the smaller tubers and in those of more spheroidal shape. The nitrogen content calculated to the fresh basis gives more significant coefficients than the nitrogen on the dry basis.

Groups A, B, and D, as well as group E again, were treated in a different way. The samples in each group were arranged in order of decreasing length-width ratio. Each group was then divided arbitrarily into two classes, one of high ratio and one of low. In each class the average value was calculated for each ratio, for the dry matter, and for the nitrogen content. These data are presented in table II. The class of high length-width ratio is termed for convenience the "long" class and the other the "short."

Although the number of samples in each class is small, comparisons will bring out general tendencies. Thus, the average width-depth ratios of the "long" and the "short" classes are not different, as indicated by the figures +2, 0, 0, and +1. The length-depth ratio seems to follow the length-width, however, as the differences between the two classes are −10, −6, −13, and −4 per cent. The dry matter is about the same for both "long" and "short" classes. The nitrogen on the fresh basis is consistently greater in the "short" classes. On the dry basis the tendency is less.

In table III the data are similarly arranged, but this time in respect to the width-depth ratio. Those of high ratio are called "flat," those of low ratio, "round" for convenience. Here it is seen that the nitrogen is consistently much higher in the "round" class.

In table IV the comparisons are made with respect to the length-depth ratio. Here again the "short" class contains much more nitrogen than the "long."

In all the above tables, group E exhibited the least range in shape ratios; and this explains the fact that it showed the lowest differences among the four groups in any one comparison.

TABLE III

FOUR LOTS OF POTATO TUBERS SEGREGATED INTO "FLAT" AND "ROUND" GROUPS ON BASIS OF WIDTH-DEPTH RATIO

CLASSIFICATION	RURAL NEW YORKER	IRISH COBBLER	EARLY OHIO	EARLY OHIO
	1922	1922	1922	1923
Group	A	B	D	E
No. of samples—				
Total	14	8	11	26
"Flat" class	9	2	3	17
"Round" class	5	6	8	9
Width-depth ratio—				
Total range	1.34–1.20	1.34–1.25	1.14–1.10	1.15–1.09
Mean of "flat" class	1.27	1.34	1.14	1.14
Mean of "round" class	1.21	1.29	1.11	1.11
Difference, per cent.	−5	−4	−3	−2
Length-depth ratio—				
Total range	1.64–1.36	1.54–1.34	1.66–1.35	1.61–1.42
Mean of "flat" class	1.52	1.54	1.61	1.54
Mean of "round" class	1.44	1.39	1.50	1.52
Difference, per cent.	−5	−10	−7	−1
Dry matter—				
Total range	25.7–20.0	22.8–17.9	25.9–22.3	24.4–20.7
Mean of "flat" class	23.1	21.3	23.5	22.2
Mean of "round" class	23.9	20.9	24.2	22.6
Difference, per cent.	+4	−2	+3	+2
Nitrogen, wet basis—				
Total range	0.543–0.350	0.518–0.326	0.529–0.410	0.499–0.377
Mean of "flat" class	0.417	0.370	0.445	0.420
Mean of "round" class	0.472	0.445	0.475	0.446
Difference, per cent.	+13	+20	+7	+6
Nitrogen, dry basis—				
Total range	2.50–1.61	2.27–1.53	2.21–1.72	2.17–1.73
Mean of "flat" class	1.86	1.74	1.89	1.89
Mean of "round" class	1.98	2.07	1.97	1.98
Difference, per cent.	+6	+20	+4	+5

The deductions from tables II, III, and IV are consistent throughout with those from the coefficients of correlation in table I. Thus three varieties of tubers show the same relations among the factors studied. For this reason it appeared justifiable to pool the data from all five groups, and calculate the ordinary coefficient of correlation between the nitrogen on the fresh basis and each of the shape ratios. The results are shown in table V. These coefficients on mixed varieties indicate the same relations as those on the Early Ohio alone: no significant value as regards length-width, but

TABLE IV

FOUR LOTS OF POTATO TUBERS SEGREGATED INTO "LONG" AND "SHORT" GROUPS ON BASIS OF LENGTH-DEPTH RATIO

CLASSIFICATION	RURAL NEW YORKER	IRISH COBBLER	EARLY OHIO	EARLY OHIO
	1922	1922	1922	1923
Group	A	B	D	E
No. of samples—				
Total	14	8	11	26
"Long" class	7	2	6	14
"Short" class	7	6	5	12
Length-depth ratio—				
Total range	1.64–1.36	1.54–1.34	1.66–1.35	1.61–1.42
Mean of "long" class	1.58	1.54	1.63	1.57
Mean of "short" class	1.41	1.39	1.41	1.49
Difference, per cent.	−11	−10	−13	−5
Dry matter—				
Total range	25.7–20.0	22.8–17.9	25.9–22.3	24.4–20.7
Mean of "long" class	23.6	21.3	24.3	2.21
Mean of "short" class	23.3	20.9	23.8	2.26
Difference, per cent.	−1	−2	−2	+2
Nitrogen, wet basis—				
Total range	0.543–0.350	0.518–0.326	0.529–0.410	0.499–0.377
Mean of "long" class	0.405	0.370	0.439	0.423
Mean of "short" class	0.469	0.445	0.479	0.435
Difference, per cent.	+16	+20	+9	+3
Nitrogen, dry basis—				
Total range	2.50–1.61	2.27–1.53	2.21–1.72	2.17–1.73
Mean of "long" class	1.75	1.74	1.88	1.91
Mean of "short" class	2.06	2.08	2.03	1.93
Difference, per cent.	+18	+20	+8	+1

marked negative correlation as regards length-depth and width-depth. This emphasizes again that depth is the determining dimension so far as nitrogen content is concerned.

TABLE V

COEFFICIENTS OF CORRELATION BETWEEN NITROGEN CONTENT AND SHAPE RATIOS IN POTATO TUBERS

$n = 67$

	$\frac{\text{LENGTH}}{\text{WIDTH}}$	$\frac{\text{LENGTH}}{\text{DEPTH}}$	$\frac{\text{WIDTH}}{\text{DEPTH}}$
Nitrogen (fresh basis)	$+ 0.0959 \pm 0.0816$	$- 0.2957 \pm 0.0752$	$- 0.4223 \pm 0.0677$

Conclusions

When the shape ratios of Early Ohio potato tubers are compared among themselves, it is found that there is a marked positive correlation between length-width and length-depth, and between length-depth and width-depth, but no relation between length-width and width-depth. Thus the length is the dominating dimension. It is also the dominating dimension when the shape ratios are compared with the size of tuber, indicating that as the tubers grow in size it is largely because of increased length. The nitrogen content of the tubers is governed more by the depth than by any other dimension. The smaller tubers tend to have a higher nitrogen content than the larger. These conclusions hold whether the comparisons are made within a variety, or among samples of tubers from several varieties.

If a variety of potato of high protein content be sought, it is believed that it will more likely be found in one having moderate sized tubers of spheroidal shape.

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BRIEF PAPERS

THE WATER CONTENT OF WHEAT LEAVES AT FLOWERING TIME

ANNIE M. HURD-KARRER AND J. W. TAYLOR

(WITH ONE FIGURE)

When wheat plants are grown under certain constant growth conditions it has been reported[1] that the water content of their stems and leaves increases to a maximum at flowering time and decreases suddenly one or two days after the stamens have extruded. As an explanation the theory has been advanced that "the imbibition-capacity of the plasma colloids, which through the action of the growth-hormones is kept up as long as a vegetative growing point is present, decreases as soon as, through the act of fertilization, this growing point is virtually lost to the vegetative plant." As the suggestion is made that the results are of significance in connection with practical problems of irrigation during the flowering period, some determinations of the moisture content of normally developing field plants during this period may be of interest.

Dry-weight determinations were made at intervals of two or three days during the flowering and early maturation periods of plants of two fall-sown wheat varieties, Kanred and Purplestraw, which flowered and ripened on different dates. In the case of Purplestraw, there was a difference of six days in the time of flowering of two adjacent plots, the seed for which had been sown on different dates. This gave an opportunity to compare on the same days the moisture content of plants of one variety in different stages with reference to flowering.

Twenty primary tillers from each of the three plots, Kanred, Purplestraw no. 1, and Purplestraw no. 2, were cut close to the ground about every other morning, wrapped in paper to lessen evaporation, and taken to the laboratory, a short distance from the field on Arlington Experiment Farm, Rosslyn, Virginia. As rapidly as possible all the leaves were cut off at the ligule and those of each position were weighed separately.[2] Any external moisture present was removed by careful wiping with cheesecloth.

The material was dried at 100° C. for three days, by which time it reached practically constant weight. The procedure recommended by SANDE-BAKHUYZEN to eliminate sampling errors by dividing the values for the leaves by the leaf area was not followed. It has been found impossible

[1] SANDE-BAKHUYZEN, H. L. Studies upon wheat grown under constant conditions. Plant Physiol. 3: 1–30. 1928.

[2] The leaves were numbered from the base of the plant upward.

TABLE I

ABSOLUTE MOISTURE CONTENTS AND PERCENTAGES OF DRY MATTER OF LEAVES OF FIELD-GROWN WHEAT PLANTS DURING THE FLOWERING AND MATURATION PERIODS[a]

DATE (1928)	GREEN WEIGHT OF 20 LEAVES (IN GRAMS)				MOISTURE IN 20 LEAVES (IN GRAMS)				DRY WEIGHT (PERCENTAGES)			
	1ST LEAVES	2ND LEAVES	3RD LEAVES	4TH LEAVES	1ST LEAVES	2ND LEAVES	3RD LEAVES	4TH LEAVES	1ST LEAVES	2ND LEAVES	3RD LEAVES	4TH LEAVES
	Kanred											
May 22	(2.85)	6.26	4.75		(2.00)	4.79	3.48		(29.82)	23.48	26.74	
24	[1.65]	(5.59)	4.75		[0.87]	(4.20)	3.39		[47.27]	(24.87)	28.63	
26	[1.15]	(4.60)	4.11		[0.51]	(3.47)	2.89		[55.65]	(24.57)	29.68	
28	[1.04]	(4.15)	3.96		[0.40]	(3.09)	2.82		[61.54]	(25.54)	28.79	
31*	[1.09]	[3.64]	4.71		[0.33]	[2.39]	3.29		[69.72]	[34.34]	30.15	
June 2	[0.91]	[2.20]	3.48		[0.22]	[1.20]	2.41		[75.82]	[45.45]	30.75	
5	[0.71]	[1.72]	3.21		[0.15]	[0.93]	2.25		[78.87]	[45.93]	29.91	
	Purplestraw no. 1											
May 22	(4.24)	5.67	7.28	5.26	(3.45)	4.50	5.63	3.86	(18.63)	20.63	22.66	26.62
24	(3.57)	5.11	6.96	5.05	(2.79)	3.94	5.25	3.55	(21.85)	22.90	24.57	29.70
26*	(3.24)	(4.91)	5.91	4.30	(2.48)	(3.81)	4.47	2.97	(23.46)	(22.40)	24.37	30.93
28	(3.20)	(5.03)	6.43	4.50	(2.41)	(3.89)	4.83	3.14	(24.69)	(22.66)	24.88	30.22
31	(3.11)	(4.56)	6.25	4.47	(2.27)	(3.38)	4.59	3.02	(27.01)	(25.88)	26.56	32.44
June 2	[1.57]	[3.10]	(5.85)	4.46	[0.93]	[2.13]	(4.27)	2.95	[40.76]	[31.29]	(27.01)	33.86
5	[1.48]	[3.02]	(6.24)	4.69	[0.85]	[1.80]	(4.98)	3.27	[42.57]	[40.40]	(20.19)	30.28
	Purplestraw no. 2											
May 22*	(3.92)	4.86	3.96	2.33	(3.02)	3.75	2.96	1.64	(22.96)	22.84	25.25	29.62
24	(3.35)	(4.28)	3.68	2.32	(2.37)	(3.13)	2.65	1.57	(29.25)	(26.87)	27.99	32.33
26	(2.93)	(4.21)	3.82	2.28	(2.02)	(3.05)	2.72	1.51	(31.06)	(27.55)	28.80	33.77
28	(3.01)	(4.25)	(4.10)	2.51	(2.07)	(3.07)	(2.92)	1.67	(31.23)	(27.76)	(28.78)	33.47
31	[1.98]	[3.07]	(3.15)	2.12	[1.24]	[2.20]	(2.24)	1.38	[37.37]	[28.34]	(28.89)	34.91
June 2	[1.50]	[3.42]	(3.17)	2.08	[0.84]	——	(2.21)	1.35	[44.00]	——	(30.28)	35.10

[a] An asterisk denotes date of flowering; parentheses, leaves partially yellow or dried; brackets, leaves completely withered.

to measure leaf area accurately during the maturation period after the leaves have begun to shrivel. Moreover, the leaves of each position from selected primary culms were fairly uniform in size, and individual variations in the rate of withering undoubtedly caused as much or more irregularity in the data than did variations in size. When as many as twenty leaves were taken for one sample, neither the small variations in total leaf area nor differences in degree of withering obscured the trends shown by the dry-weight measurements.

There is no evidence in these data of a maximum water content just before flowering nor of a sudden water loss after flowering. In general the water content of the leaves of both varieties was decreasing from the day the experiment was begun, which was nine days before flowering in the case of Kanred, and four days before in the case of Purplestraw no. 1. It is true that a relatively rapid decrease occurred just after flowering in the second and third leaves of Kanred, but a similar decrease occurred on the same dates in the lower leaves of Purplestraw no. 1, which was at that time seven days past flowering (fig. 1). Therefore the decrease in both varieties was apparently due to the warm, dry weather which prevailed at this time.[3]

The dry-weight percentages also give no evidence of a relation between the rate of drying and the date of flowering of these plants. In the case of Kanred, the lowest leaves were almost completely withered, and the second leaves partially withered, before flowering time. There was no significant change in the water content of the upper leaf, which remained green throughout the experiment. In the case of Purplestraw no. 1, there was no sudden increase in any of the leaves immediately after flowering. In Purplestraw no. 2, the percentages increased slightly for all leaves just after flowering, but the upward trend probably began before the first measurement, which was made just at flowering time. Furthermore, a similar increase occurred over the same period in the plants of the other two plots (excepting the completely withered first leaf of Kanred) and these plots were in pre-flowering stages.

SANDE-BAKHUYZEN states that in his experiments the first sign of the water loss after flowering was the yellowing of the tips of the leaves. Under field conditions yellowing of the leaves of wheat plants often begins before flowering, the time depending on environmental conditions and on the adaptability of the variety to the environment. In the present experiments the tips of at least the first leaves yellowed before flowering time in each of

[3] The temperature and rainfall records were taken by Mr. A. MAYER at Arlington Farm, and are for 24-hour periods ending at 8:00 a. m. each day. Since the plants were always cut between 9 and 11 o'clock in the morning, the figures given in the graphs refer in each case to the 24-hour period preceding the date of sampling.

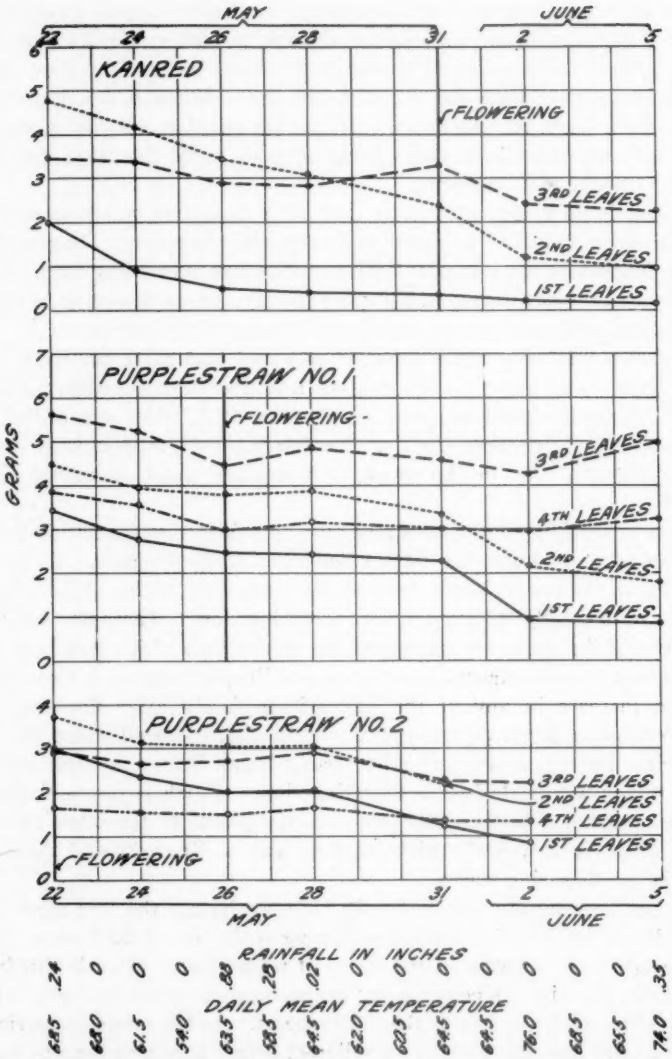

FIG. 1. Moisture in 20 leaves of each position on primary tillers of field-grown wheat plants during the maturation period.

the three plots. The lowest leaves of Kanred were completely withered seven days before flowering and the tips of the second leaves had begun to yellow six days before. In the case of Purplestraw, also, the first leaves were all beginning to turn yellow before the plants flowered.

Since the water content of the field plants fluctuated somewhat with changes in the external environment, some measurements were made on greenhouse plants grown under fairly uniform temperature and moisture conditions. It was possible to obtain tillers in pre-flowering, flowering, and post-flowering stages at one time and in the same rows. The data showed no differences in moisture content which were correlatable with differences in the stage of development of the tillers. Consequently, it appears that the physiological processes involved in flowering did not exert any specific effect on their water content.

Of course, the results of these experiments do not preclude the possibility that under a given set of conditions, such as those of a constant environment, desiccation might always begin immediately after flowering.

OFFICE OF CEREAL INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

NOTES

Annual Election.—The results of the annual election of the American Society of Plant Physiologists has been announced by the Secretary-Treasurer, Dr. H. R. KRAYBILL, as follows: For president, Dr. SCOTT V. EATON, of the University of Chicago; for vice-president, Prof. CHARLES A. SHULL, also of the University of Chicago. Because of previous service, the vice-president elect declined to serve, and the executive committee has elected Dr. A. E. MURNEEK, of the University of Missouri, to the vice-presidency. The new officers will have the hearty support of the entire membership. The growth of the Society during the last year has been very gratifying, and the financial condition at the close of the fiscal year on June 30 was stronger than at any time in the last four years.

Program Committee.—The program committee for the Des Moines meeting was appointed some time ago by the retiring president, Dr. E. J. KRAUS, of the University of Chicago. The committee is constituted as follows: Dr. W. E. LOOMIS, chairman, Iowa State College; Dr. J. P. BENNETT, Ohio Agricultural Experiment Station; Prof. L. F. GRABER, the University of Wisconsin; and Dr. H. R. KRAYBILL, of Purdue University, *ex officio*. All members of the Society are reminded of the desirability of concentrating our efforts on the Des Moines meeting. The program committee will appreciate the cooperation of all members, and each one should be prepared to respond promptly for any service that may be requested. With proper efforts the Society can greatly exceed any previous meeting in interest and enthusiasm.

Finance Committee.—The new constitution provides for a finance committee whose duties as stated are "to act in an advisory capacity to the Secretary-Treasurer with reference to the productive funds of the Society. The committee shall also seek ways and means of increasing the productive funds for support of the Society's work." The committee appointed by the President for this service includes Prof. CHARLES A. SHULL, chairman; Dr. WALTER THOMAS, Pennsylvania State College; and Dr. BURTON E. LIVINGSTON, Johns Hopkins University. The committee is appointed for three years, and the members will take their duties seriously. They will appreciate constructive suggestions at any time. An active program in furtherance of the purposes stated in the constitution will be initiated.

Life Membership Committee.—The selection of the fourth recipient of the CHARLES REID BARNES Life Membership in the American Society of Plant Physiologists has been entrusted by President SCOTT V. EATON to a

committee of members as follows: Dr. WALTER THOMAS, chairman; Dr. EARL S. JOHNSTON, University of Maryland; Dr. J. D. SAYRE, Ohio Agricultural Experiment Station; Dr. R. B. DUSTMAN, West Virginia University; and Prof. J. H. GOURLEY, Ohio Agricultural Experiment Station.

Portraits of Wilhelm Pfeffer.—As in the case of the portraits of SACHS and TIMIRIAZEFF, a number of portraits of PFEFFER have been printed without the plate number and name of the journal, to provide portraits suitable for framing. These copies are obtainable from the editor of PLANT PHYSIOLOGY at 12 cents each, postage prepaid. Stamps may be sent in payment.

Fall Meeting, American Chemical Society, Division of Colloid Chemistry.—The Division of Colloid Chemistry is to hold two half-day sessions at the fall meeting of the American Chemical Society in Minneapolis Sept. 9–13, 1929. The arrangements for the meeting are in the hands of Dr. F. E. BARTELL, of the Chemistry Department, University of Michigan, who is Chairman of the Division, and Dr. ROSS A. GORTNER, of the Division of Agricultural Biochemistry, University of Minnesota, Secretary-Treasurer of the Division of Colloid Chemistry. These meetings are valuable to anyone interested in the behavior of colloidal materials, and plant physiologists particularly would find them profitable.

International Horticultural Congress.—The ninth International Horticultural Congress will be held in London, England, from August 7 to August 15, 1930, just preceding the fifth International Botanical Congress, which is to be held at Cambridge, August 16–23, 1930. The Secretary of the Royal Horticultural Society, Mr. F. R. DURHAM, is secretary of the congress. The cost of membership in the congress will be one pound sterling, and should be sent to the Secretary of the Royal Horticultural Society, Vincent Square, London, S. W. 1. The main topic for discussion will be "Propagation, vegetative and seminal." An extensive program for visits to research stations and gardens of horticultural interest throughout the United Kingdom is being arranged. It will be a rare privilege for those planning to go to England in 1930 to attend both of these International Congresses.

Purdue Section.—It is a pleasure to record the progress of the Purdue Section of the Society. Their work has long since passed the experimental stage, and the value of sectional activity has been abundantly demonstrated. The last year was one of unusually interesting programs. The Section's meetings were held twice a month under the chairmanship of Dr. C. L. PORTER. The program was initiated with a dinner meeting at which Dr. ENDERS gave an interesting review of "Some recent advances in general physiology." The program was terminated with an address by Dr.

CHARLES F. HOTTES, Head of the Department of Botany, the University of Illinois. Dr. HOTTES chose as his subject for this address "The physiology of the cell." A group of fifty or more enjoyed his talk, dealing with recent research in relation to earlier work on the cell.

The year's program was divided in such manner that the first semester was devoted to a study of the nutrition of the higher plants, and the second semester to the nutrition of the fungi. The papers presented were as follows:

October 15, 1928, Some recent advances in general physiology. Dr. H. E. ENDERS.

November 5, Nitrogen synthesis of higher plants. Dr. R. H. CARR.

November 19, Recent studies on carbohydrate synthesis. Dr. R. E. GIRTON.

December 3, Baron von Liebig's laboratory. Mr. H. F. HULSEMANN.

January 7, 1929, Joint evening meeting with the Biological Society. Some papers of interest at the New York meeting of the A. A. A. S.

January 14, Iron in plant nutrition. Mr. J. F. TROST.

February 4, Spore germination. Factors influencing germination, especially nutritive compounds, host influence, etc. Mr. COMPTON.

February 18, Relation of vegetative growth to nutrition of saprophytes. Mr. SAMPSON.

March 4, Establishment of nutritive connection in parasites (infection). Mr. DOAK.

March 18, Relation of parasites to the metabolism of their hosts. Effect of mineral nutrition, temperature, etc. Mr. WHITE.

April 1, Nature of resistance to parasites. Dr. E. B. MAINS.

April 15, Physiology of the cell. Dr. CHARLES F. HOTTES, University of Illinois.

Dr. R. H. CARR was elected chairman, and Dr. R. E. GIRTON secretary for the year 1929–1930.

Stephen Hales.—Admirers of STEPHEN HALES can now obtain an attractive biography of this interesting plant physiologist and natural philosopher. The biographer is Dr. A. E. CLARK-KENNEDY, Fellow of Corpus Christi, Assistant Director of the Medical Unit, and Assistant Physician to the London Hospital. The story of HALES's life and works is told in thirteen intensely interesting chapters, with fourteen illustrations. The frontispiece is a portrait of HALES, from the painting by Hudson in the National Gallery, London. Other illustrations show the Corpus Christi College which HALES attended, STUKELEY's map of the countryside around Cambridge and the Gogmagog Hills, where he and HALES botanized together, STUKELEY's drawing of HALES's orrery, a portrait of STUKELEY, a few plates of reproductions from Vegetable Staticks, Newgate prison,

equipped with a ventilator invented by HALES, and the parish church in Teddington where HALES served the spiritual needs of his people.

Every plant physiologist should know something of the historical background of plant physiology. HALES was the first great modern experimentalist in the field of plant physiology. The American Society of Plant Physiologists celebrated the 250th anniversary of his birth, and the 200th anniversary of his Vegetable Staticks at the Nashville Meeting in December, 1927, at which time a prize fund was established in honor of HALES.

The biography is an intimate account of a great scientist and a great humanitarian. The book is published by the Cambridge University Press, at $6.00 per copy. Orders in this country should be sent to the Macmillan Co., New York.

Botany.—The science of Botany is being well served so far as the number of available new text-books is concerned. A recent addition to the literature of botany is the text by Dr. W. J. ROBBINS and HAROLD W. RICKETT, of the University of Missouri, entitled Botany, a text-book for college and university students. The approach to the subject is physiological. After a brief chapter on the cellular structure of plants, there are eleven chapters covering absorption of water and dissolved materials, the functions of roots and stems, leaf functions, food, energy relations, formation of new cells, growth, reactions to stimuli, life and death, and the origin of life.

The next twelve chapters consider the various groups of plants, beginning with the bacteria, yeasts, molds, and fungi. There are chapters on algae, ferns, bryophytes, club mosses, pine, and angiosperms (two chapters). The final chapters are on inheritance, evolution, and distribution. Review questions occupy 37 pages at the close of the text, and an appendix contains 101 titles, mainly of books, for reference. Only a few citations to periodic literature are included in the list.

The text is simply and clearly written. It seems to the reviewer a good beginning text for the general course in botany. Some of the topics are possibly treated in too elementary fashion for college and university students, particularly if they have had a year of botany in high school. It is better, however, to err on the side of simplicity than to be too technical in the first year college text. Teachers will find the book actually practicable in use, and students will find it interesting and stimulative. The price is $3.75. Orders for the text should be sent to D. Van Nostrand Co., New York. A book of laboratory instructions by the same authors may be had to run along with the text.

The Physiological Basis of Drought Resistance.—Three years ago there was noted in these columns, under this title, the book which Prof. N.

A. MAXIMOV had just produced, and its forthcoming translation from Russian into English was announced. Owing to the illness of the editor, Prof. YAPP, this work was greatly delayed, and has only just appeared, with the title, The Plant in Relation to Water, and the subtitle as above. Prof. YAPP lived only long enough to see the last proof sheets, and never saw the book he edited from a sick-bed. The general plan of the book is unchanged from the Russian original, although the excellent account of sap ascent available in English permitted the elimination of chapters dealing with this, and those on cellular absorption have been condensed. Part I contains 75 pages, and treats in three chapters of The Absorption of Water by the Plant. Part II has 118 pages, and devotes four chapters to The Loss of Water from the Plant. Part III covers 184 pages and contains five chapters on The Water Balance and Drought Resistance of Plants. While the first two parts are admirably up-to-date and adequate in their treatment, the third part is the most significant portion of the book. And of this, the last two chapters are of especial interest, as embodying MAXIMOV's own results and theories regarding the relation between xeromorphism and drought resistance. Throughout the book, one is constantly impressed with the painstaking care of the author to include American and English work, as well as the more expected Russian and German work, and with the critical analysis given methods and results. This is no mere compilation, but a monograph by a recognized authority who has himself worked at the problem for fifteen years at first hand, and it deserves a wide reading by plant physiologists everywhere, both academic and applied. We owe a debt of gratitude to the author and the late editor for making available in concise form all the widely scattered data bearing on the water relations of plants, and especially the great amount of normally unavailable Russian work. It is a pity that so valuable a book is so poorly bound. There are 400 pages of text, a bibliography of 558 citations, and a good index. The publishers are Allen & Unwin, London, and the price from Macmillan Co., $9.00.

Fixation of Atmospheric Nitrogen.—A book of considerable interest to the biologist has been published on this subject by FRANK A. ERNST, of the Fixed Nitrogen Research Laboratory, U. S. Department of Agriculture. The book is concerned mainly with the commercial fixation of nitrogen, a chapter being devoted to each of the three main commercial processes, namely, the arc process, the cyanamide process, and the direct synthetic ammonia process. Equations showing the chemical reactions involved in these processes, and the energy relations of the reactions are given. Other chapters consider the various ammonia conversion products; economic considerations that are leading to the gradual replacement of Chile nitrate by the products of the commercial processes, and to the supremacy of the syn-

thetic ammonia process among the various commercial processes; statistics of the production of Chile nitrate and of the world production of inorganic nitrogen by the commercial processes. There are appendices giving the cost of production of Chile nitrate, the location of the world's plants for the fixation of atmospheric nitrogen, with annual capacity and 1927 rate of production, and nitrogen statistics of production, export, import and consumption for the principal countries of the world. The book contains 154 pages and is published by D. Van Nostrand Co., New York. The price is $2.50.